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THE HISTOGENESIS AND GROWTH OF THE OTIC CAPSULE AND ITS CONTAINED PERIOTIC TISSUE-SPACES IN THE HUMAN EMBRYO.

By George L. Streeter.

With four text-figures and four plates.

CONTENTS.

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By George L. Streeter

INTRODUCTION.

During the past year the writer has published two shorter communications regarding the development of the connective tissue and cartilaginous coverings that inclose the membranous labyrinth, one of which dealt with the histogenesis of the cartilaginous capsule and the other with the periotic tissue-spaces that are formed in the interval between the cartilaginous capsule and the membranous labyrinth. In the present paper the same matter will be treated in more complete form and a general description will be given of the development of the otic capsule as a whole and of the problems involved in its growth.

In making this study the effort has not been so much toward the determination of the exact form of the capsule as it has been toward the detection of some of the factors that are involved in the production of this form. These two problems, however, are not to be altogether separated. It is the distinctive form of the otic capsule that makes it a particularly favorable place for determining the histological features of the growth of such a structure. Owing to the fact that it is so well provided with known landmarks, the changes in its size and form can be accurately followed and it is therefore possible to determine deductively at what points, for instance, new cartilage is being laid down and at what points it is being removed.

It was soon recognized that the growth of the otic capsule resolves itself into an increase in its external dimensions and a simultaneous hollowing-out and reshaping of its contained cavities, the latter being so managed that their general form and proportions are continuously maintained and a suitable space always provided for the enlarging membranous labyrinth. It is particularly the feature of cartilage excavation accompanying the increase in the total mass to which attention will be invited. It is quite evident that such growth can not be explained on the basis of a simple interstitial increase in the amount of cartilage, together with its passive rearrangement to allow for the enlarging cavities, due, one might say, to a mechanical expansive pressure from the growing membranous labyrinth with its surrounding tissue and fluid. Such a passive rearrangement could only occur in a tissue that is very plastic, whereas cartilage is one of the least plastic of the embryonic tissues. Moreover, the histological picture is not that of mechanical pressure; the cartilaginous chambers are always excavated slightly in advance of the space actually required by the membranous labyrinth, and there is no evidence of the labyrinth being cramped or of the creation of pressure grooves in the margin of the cartilage.

Furthermore, it can not be the perichondrium that is the essential factor, either in the deposit of new cartilage or in the excavation of the old, because the perichondrium, as we shall see, is not formed until after a considerable amount of the growth and hollowing-out of the labyrinth is already completed. Therefore, in the development of the cartilaginous capsule there is something more than interstitial and perichondrial growth.

As forming at least one element, and an important one, in this process it has been found that there occurs a regression of certain areas of cartilaginous tissue to a more embryonic form followed by its alteration into a different type of tissue. It is this process of dedifferentiation that constitutes the essential factor in the hollowing-out and reshaping of the otic capsule which take place continuously during its development. Though the significance and wide occurrence of dedifferentiation and redifferentiation have been well known to botanists and to those investigators who have worked with the simpler forms of animal life, this, as far as the writer knows, is the first time that they have been shown to occur in the human embryo. It is not unlikely that these principles will eventually enter into our conception of the growth of other tissues and organs in human as well as in other mammalian embryos. The establishment of this point, of the occurrence of retrogressive as well as progressive differentiation in human embryos, is considered by the writer to be the chief contribution of the following paper.

The fate of the periotic connective tissue that intervenes between the cartilage and the membranous labyrinth and the formation of the characteristic periotic spaces form problems that are naturally of a morphological character. These spaces have been studied by modeling methods and a description will be given of the steps by which the larger spaces acquire their adult form. It will be pointed out that these spaces show a marked individuality. They have constant and definite characteristics, including their time and point of origin, the manner in which they spread, and their eventual form and structure. They have a structural individuality which, though less complicated, is just as definite as that of the other parts of this sense-mechanism. All of this we will come to later.

TERMINOLOGY.

The writer is not unmindful of a certain feeling of distress that is aroused when it is found on reading a new paper that the author of it is adding to the already difficult matter of following another's description by making a new application of terms or by introducing a whole battery of freshly created ones. Nomenclature constitutes one field in which rock-bound conservatism has many points of merit and where originality may expect a cold and critical reception. It is therefore with some embarrassment that the writer approaches the subject of terminology, and it is also with some apprehension as to whether the "originality" in this instance will prove to be justified. It has in fact seemed best to avoid the incorporation of the term "lymphatic" in describing of the tissue-spaces surrounding the membranous labyrinth. It has been the custom to designate these as "perilymphatic" spaces since 1833, when the term was introduced by Breschet, who thus distinguished

them from the "endolymphatic" cavities of the membranous labyrinth. These terms, together with the terms "perilymph" and "endolymph" for their contained fluids, seemed particularly appropriate and in practical use have proved to be very convenient. Since Breschet's time, however, the lymphatic vascular system has taken on an increased and individual importance, due to researches in which American investigators have taken a particularly active part, and it now seems important to restrict the term "lymphatic" to it and its associated structures.

Inasmuch as the tissue-spaces surrounding the labyrinth have no known connection with the true lymphatic system, either in their origin or in their ultimate relations, it follows that the use of the term "lymphatic" in connection with them is misleading. It therefore seems advisable to eliminate it, even at the expense of losing such a convenient terminology. As a substitute for "perilymphatic" the term "periotic" was finally decided upon and will be so used throughout this paper. In the formation of this adjective the Greek word οὖς, from which it is derived, is used in the restricted sense of representing the essential sense-organ, that is, the otocyst itself and eventually the membranous labyrinth. Inasmuch as numerous words derived from the same source are in common use, it is felt that this term will be readily understood.

We shall speak of a periotic connective tissue that everywhere surrounds the membranous labyrinth. This periotic connective tissue includes in part the fine-meshed periotic reticulum, and in part the large walled-off periotic spaces with their contained periotic fluid, the most prominent of which are the scala vestibuli, scala tympani, and the vestibular cistern. For the term "endolymphatic" fluid one could substitute "otic" fluid; we would then have "liquor perioticus" and "liquor oticus." In all other instances, except when elsewhere specified, the Basiliensis Nomina Anatomica terms have been adhered to. The term "semicircular duct" is used to specify the epithelial or membranous canal as distinguished from the cartilaginous semicircular canal. This usage was recommended by Breschet and was adopted in the BNA. It was not taken advantage of, however, by Retzius (1884) in his monograph on the vertebrate ear, who used the term "semicircular canal" for the epithelial channel as well as for the cavity in which it lies. The influence of this great monograph has delayed somewhat the adoption of the BNA recommendation, and one finds subsequent writers still following Retzius in this respect, among whom may be mentioned v. Ebner, R. Krause, Röthig, and myself in previous papers on the development of the membranous labyrinth. In a similar manner the usage by Retzius of the term "anterior" canal instead of "superior" canal, as recommended by the BNA, has occurred in relatively recent papers, including, it must be confessed, those of my own. In the present paper, however, this usage has been corrected. In the historical review which follows the various structures mentioned will be largely referred to in the older terms used by the respective authors.

HISTORICAL.

The first monographic treatise on the anatomy and physiology of the ear was that published by Breschet in 1833. This work proved to be a very important one, both as regards the new observations contained in it and the constructive manner in which the facts then known were analyzed. The terminology of the ear region was standardized and most of the names that were used or introduced by Breschet are in use in the literature of to-day. Before stating his views concerning the structures with which we are dealing, reference must be made to the work of some of his predecessors, and this will be given essentially as outlined by him.

The early anatomists were familiar with the bony labyrinth, but supposed that the spaces contained within it were filled with air. In 1707, however, Valsalva described the normal presence of a fluid in the labyrinth which he compared to the fluid seen in serous cavities. The presence of this fluid was confirmed by Vieussens (1714). His observations were made chiefly on new-born infants, in which he studied the distribution of the labyrinthine fluid and found it present in the vestibule, the cochlea, and in the semicircular canals. The same fluid was also referred to by Cassebohm (1735) and Morgagni (1740). Up to that time no author had directed any particular attention to the labyrinthine fluid, nor had anyone attempted to assign any function to it other than that of moistening the auditory nerve. It was Cotugno (1768) who first endeavored to show that the labyrinthine fluid had some connection with the transmission of sound vibrations. He maintained that there was no air in the spaces of the labyrinth, but that it was everywhere filled with the fluid, which according to his description exudes from the ends of the capillary arteries that are distributed throughout the membrane that lines the cavity of the labyrinth. He described the fluid as being drained off by means of the two aqueducts. Because of the completeness of his description and the interest which he attracted toward the subject, the labyrinthine fluid was thereafter known for more than half of a century as Cotugno's fluid.

Any further advance regarding the nature of the labyrinthine fluid required a more detailed knowledge of the soft parts of the labyrinth. Nerve-like cords and semicircular tubes had been seen in the canals and membranous partitions and sacs had been seen in the vestibule, but it remained for Scarpa (1789) to establish the identity of the membranous labyrinth. He showed that in man and other mammals the semicircular tubes and the vestibular sacs are of the same nature and form one system, and that they are distinct from periosteum. He described how they open freely into each other and are filled with a limpid fluid which distends them. This fluid was thereafter referred to as the fluid of Scarpa. He recognized it in a general way as distinct from the labyrinthine fluid, in which all parts of the membranous labyrinth floated, but otherwise grants it no further attention.

The relations and significance of the fluid of Cotugno and the fluid of Scarpa were not completely recognized until the publication of the noteworthy monograph of Breschet (1833) of which we have spoken. He introduced the terms perilymph and endolymph, by which they have since been known. The existence of the cochlear duct was unknown to Breschet, but otherwise his description of the labyrinth spaces and their contained fluids is the foundation on which the more recent descrip-

tions are principally based. He showed that the perilymph occupies all the space of the bony labyrinth that is not taken up by the semicircular tubes, the utricle (median sinus), and the saccule. It surrounds these everywhere and separates them from the bony walls of the labyrinth. The perilymph also, according to him, fills the spaces of the cochlea and circulates freely throughout the whole system. The scala tympani is connected at its apical extremity with the scala vestibuli by means of the opening to which he gave the name helicotrema. The scala vestibuli in turn opens freely into the vestibule, into which also the semicircular tubes open. He points out the fact, and discusses its relation to the mechanism of hearing, that any vibrations transmitted to the perilymph by the foot-plate of the stapes would be transmitted freely and evenly to the whole of the membranous labyrinth and to the lamina spiralis. He describes the perilymph as consisting of a thin, watery, saline fluid containing a small amount of albumin. He believed that it was secreted by the thin, delicate membrane lining the cavity of the labyrinth and that the materials were brought there by the small blood-vessels that supply this layer. The aqueducts, according to him, are not for the transmission of perilymph, but only serve as passages for veins. He regards them of embryological significance; that they represent the remaining strands of connection with the dura mater, of which the inner ear is a part that has been separated off by the enveloping growth of bone. A description is given of the distribution of perilymph in different animals and it is pointed out that in some fish it communicates with the fluid surrounding the central nervous system and how in such cases the oily or gelatinous cerebro-spinal fluid actually serves as the perilymph. Entirely distinct from the perilymph is the endolymph, which is the fluid filling all parts of the membranous labyrinth.

Breschet describes the character of the endolymph in different animals. He shows that it always contains calcareous deposits, which he designates as otoliths and otoconia, depending on whether they are in the form of lumps or dust. He shows that these are distributed at definite places, at the points of nerve-terminations, and suggests that they act as dampers that tend to check the prolonged vibration of the endolymph. In comparing the ear with the eye, he suggests that the perilymph bears the same relation to the organ of hearing that the aqueous humor bears to the organ of vision. The vitreous body he considers analogous to the endolymph.

Special emphasis has been given to the treatise of Breschet because it marks the beginning of the modern epoch in the anatomy of the ear. Previously the descriptions of this organ had been purely fragmentary. Breschet's monograph is both comprehensive and analytic. If his treatise is searched for defects that are revealed in the light of our present knowledge of the anatomy of the ear, one would name perhaps only two major ones. One of these concerns the inaccurate and meager nature of the embryological features as given by him, and the other concerns the membranous cochlea, the existence of which was entirely unknown at that time. It is interesting to note that these two defects prove to be related; that it was through embryological investigations that the membranous cochlea was eventually discovered.

At about the time of Breschet's treatise, Huschke (1831) made the discovery that the membranous labyrinth was originally a pit in the skin, a fundamental point

that was confirmed later by other embryologists. He also found that in sheep and calf embryos the lamina spiralis is hollow, constituting a spiral tube that is closely attached to the bony walls of the cochlea. He evidently had before him the ductus cochlearis. He supposed, however, that the scalæ in their formation flattened out this hollow tube, thereby converting it into the lamina spiralis, and thus he did not grasp the meaning of the structure and just missed being the discoverer of the membranous cochlea.

The significance of the spiral tube seen by Huschke in the embryo and its persistence as the ductus cochlearis in the adult remained to be pointed out by Reissner and Reichert in a series of communications published in the years 1851 to 1854, being based in large part on embryological studies of the chick and also of mammals. The first communication was the Dorpat dissertation of the former, completed in Reichert's laboratory. It contained an account of the ductus cochlearis (canalis cochlearis), which was shown to exist as a definite canal in the adult mammalian cochlea. It was pointed out that the membranous part of the lamina spiralis forms one-half of the wall of the canal and that the other half consists of a very thin and delicate membrane that is usually torn in the preparation of such a specimen. This portion has since been known as the membrane of Reissner.

Reissner and Reichert demonstrated the complete canal in infants at about term. In the embryo they found that the cochlear duct opens into the membranous vestibule, but whether or not it does this in the adult was not definitely determined. They give an account of the development of the ear in the chick, and they describe the formation of a labyrinthine groove in the skin and how this subsequently invaginates to become the otic vesicle to which the acoustic nerve attaches itself later. They divide the early labyrinth into three chief parts: (1) recessus labyrinthi; (2) vestibule and its three canals; (3) cochlear duct. In a later paper Reissner (1854) refers to the formation of the scalæ. He explains them as two accessory cavities that are formed because the cartilage recedes from the upper and lower surfaces of the cochlear duct. He denies the existence of any communication between the vestibular cistern (Höhle des Vorhofs) and the scala vestibuli both in the embryo and adult. His observations concerning the scalæ have proved to be less important than those on the embryology of the otic vesicle and the discovery of the cochlear duct.

Kölliker (1854) had just at this time written the first edition of his "Gewebelehre" and had described at some length the finer structure of the cochlea, embodying his own and the important observations of Corti (1851). He makes mention of Reissner's dissertation, but there is no evidence that he appreciated at that time the significance of the cochlear duct. In a separate paper and in his text-book on human embryology, both of which appeared soon after this (Kölliker, 1861 *a* and *b*), he definitely establishes the existence of Reissner's membrane and that it forms the boundary of the cochlear canal. He confirmed the Reissner and Reichert embryological studies showing that the cochlear canal is originally an epithelial tube which is derived from the primitive ear-vesicle and hence from the ectoderm of the embryo. He designates the cochlear canal as the scala media, a term which persisted for many years, though its inapplicability was promptly pointed out by Reichert (1864).

Kölliker is the first to describe in some detail the formation of the otic capsule and the perilymphatic spaces. The summary here given is taken from the second edition of his book (Kölliker, 1879), in which there is some amplification of the account given in the first edition. According to him, after the otic vesicle reaches a certain degree of development it is surrounded by a delicate connective-tissue membrane and there is an outer thicker and firmer mass which takes on the nature of cartilage, which in 19-mm. cow embryos forms an integral part of the wall of the skull. In human embryos 8 weeks old the labyrinth capsule consists of true cartilage and completely fuses with the base of the skull. He expresses the opinion that the cartilago petrosa is laid down exactly in the same way as the other parts of the lateral walls of the skull and that its special characteristics, subsequently assumed, are due to the presence of the special sense-organ.

In connection with the origin of the cavities of the bony labyrinth, Kölliker draws attention to the problem of space formation in general and points out that the space in the otic capsule is of the type seen in the subarachnoid and other serous cavities. He describes how, along with the growth of the epithelial part of the labyrinth, there is also a rapid growth of its connective-tissue coverings, which soon attain considerable thickness. At the same time the periotic tissue becomes differentiated into three layers, of which the middle one soon becomes the thickest. This layer consists of a network of anastomosing connective-tissue cells (Gallertgewebe), whose rounded meshes are filled with fluid. From this there is gradually formed the cavity that surrounds the semicircular canals, the meshes becoming larger and finally coalescing. In the process of coalescence, parts of the cellular net are broken and other parts are pressed against the walls of the space, where, even in the adult, one can recognize remnants of the broken net. The same process takes place in the semicircular canals, the vestibule, and the cochlea. In the last-mentioned there are formed the two scalæ, in which, in addition to the coalescence of the spaces of the network, there is also involved a disproportionate growth in respect to the cochlear duct and the surrounding cartilage, the latter retracting from the former by virtue of its rapid growth. Kölliker's conception of this process is purely mechanical, and it hypothesizes a protoplasmic network that is entirely passive. He did not conceive of an adaptive activity on the part of the protoplasm itself by virtue of which the characteristic changes in form are brought about, as is to be described in the present paper.

Among a series of miscellaneous notes concerning the development of the mammalian labyrinth appended at the end of Kölliker's chapter, there is a reference as to the relation of the growth of the cartilage to the growth of the contained spaces, which is particularly interesting, as it shows that he had in mind one aspect of the problem with which we are concerned. He notes there (p. 746) that at first the epithelial part is directly surrounded by young cartilage, or better, a cartilage-like substance of which the greater part becomes subsequently converted into cartilage. In its further differentiation the tissue lying directly against the labyrinth becomes fibrous tissue and the tissue farther away becomes cartilage. Out of the uniform fibrous tissue there is further differentiated the inner perichondrium, the fiber wall of

the labyrinth, and the cell tissue intervening between these. This intervening tissue undergoes an independent growth characterized by a rich growth of blood-vessels. As a space, *i. e.*, the cavity of the cartilago petrosa, it grows in correspondence to the growth of the epithelial labyrinth. As to the behavior of the cartilage during the growth Kölliker was undecided, giving the opinion, however, that it grew independently at the same time as the space, and was not simply mechanically stretched out.

The embryological studies of His began to appear at about the time of the publication of Kölliker's work on the ear, and one would rather expect that the attention of this keen observer would have been attracted to this subject. In his "Akademische Programme" of the year 1865 he outlines (His, 1903) the general problem of the formation of the various body-cavities and describes in detail the formation of the cavities of the middle germ-layer. He includes in this the arachnoid spaces and the cavities of the eye-ball, but he does not refer to the ear.

Inasmuch as the present paper is directly concerned only with the capsule of the ear and the contained periotic connective-tissue spaces, it will not be necessary to trace the further elaboration of a more precise knowledge of the structure of the membranous labyrinth which rapidly took place following the appearance of Köliker's text-book and the introduction of the new histological and embryological methods which were devised in such abundance at about that time. A complete survey of such investigations is given in the monograph of Retzius (1884), to which the reader is referred. Our review here of the subsequent literature will be restricted to those publications having a special bearing on the periotic connective-tissue structures and the problem of their development.

The canalis reuniens was discovered by Hensen (1863) as a communication existing between the ductus cochlearis and the sacculus. This established the relation of the cochlear duct as a definite part of the closed system of the membranous labyrinth, and its complete separation from the vestibular space. Using the terminology of Breschet, it thus constitutes an endolymphatic space, whereas the scala vestibuli and scal atympani are both perilymphatic spaces. Hensen also described the aquæductus cochleæ. He regarded it as an invagination from the outer perichondrium into the cochlea by a process similar to the invagination of the aqueous humor of the eye. In the embryo it consists of a connective-tissue tube which is continuous with the primary periosteum. It splits into two limbs, the shorter one of which extends towards the round window and forms the lining of the proximal part of the scala tympani. The other limb of the membranous aqueduct extends towards the modiolus and unites with the dura mater of the acoustic nerve.

Hensen was followed by Odenius (1867) who gives a careful description of the position of the different parts of the membranous labyrinth and of the "perilymphatic" spaces surrounding them. He separates the perilymphatic space of the vestibule into two divisions based on the attachment of the utricle to the vestibular wall. The lower and chief division he names sinus perilymphaticus vestibuli. This communicates with the upper division, which surrounds the upper part of the utricle and extends along the semicircular canals. This part is narrower and is hardly

more than a cleft situated between the membranous labyrinth and the bony wall and is traversed by many trabeculæ.

A third author who could be put in this group is Boettcher (1869). He published two papers which bear upon the periotic spaces, but these are not available to the writer and resort has been had to the account of them given by Retzius (1884). He describes the formation of the scalæ in sheep embryos. They make their first appearance in embryos 70 mm. long, beginning in the first turn of the cochlea and gradually extending to the second and third. According to him there is a preliminary formation of mucus tissue in the region in which the scalæ are to appear; this then undergoes a fatty degeneration, the result being the formation of the spaces. He warns against confusing this special "Schleimgewebe" with ordinary intracapsular connective tissue and opposes Hensen's theory of its invagination from the outer periosteum. According to him (Boettcher, 1872), it arises *in loco* out of the original cellular embryonal connective tissue.

Although it was recognized that there must be a provision in the human adult ear for the renewal and drainage of the intralabyrinthine fluid, yet there was no positive evidence of how this was accomplished until the introduction of injection methods. Schwalbe (1869) found that when Berlin blue is injected into the subdural space the injection mass passes through the internal auditory meatus into the space existing between the bony and membranous labyrinth. Since he could also trace the injection mass from the subdural spaces into the lymph vessels and glands, he therefore believed that the perilymph spaces represented true lymph spaces, for which the arachnoid spaces acted as the main drainage-channels.

In order to test out the communication reported by Schwalbe between the arachnoidal spaces and the perilymphatic space, a series of injections were made by Weber (1869), who found that the injected fluid accompanied the acoustic nerve as far as the lamina cribrosa, but did not go through this. It passed rapidly, however, through the aquæductus cochleæ into the perilymphatic space of the cochlea. Later, this same investigator (Weber-Liel, 1879) invented the aspiration method by which the results were refined, and he was able to avoid the production of artificial paths which commonly result where strong pressure is necessary for the injection and which apparently had vitiated Schwalbe's experiments. He proved clearly that the aquæductus cochleæ was the primary path of communication between the perilymphatic and arachnoidal spaces, and that it consists of a free canal lined by an extension of dura mater connecting the scala tympani with the cranial cavity. He was not specific, however, as to whether the communication was with the subdural or subarachnoid space.

Key and Retzius (1875) in their extensive studies on the brain membranes, were able by injection methods to show that the spaces of the brain membranes stood in open communication with the perilymphatic space of the labyrinth, but, although they were able to trace the injection mass along the acoustic nerve, through the lamina cribrosa, and along the finer filaments of the nerve into the lamina spiralis, they were not sure of its communication there with the perilymphatic space. They showed, on the other hand, that the latter could not be injected through the

aquæductus vestibuli and seemed convinced that the main communication was through the aquæductus cochleæ as described by Weber, which view remains the prevalent one to-day.

It may be added that Retzius (1884) subsequently made some further injection experiments in older fetuses and in the adult, which were published in his large monograph on the ear. He found (p. 330) that in this way the scala tympani communicates freely through the ductus perilymphaticus with the subarachnoid spaces, and not with the subdural space, which point had been left undecided by Weber. By injecting through either the round or oval window he was able to trace the escape of the fluid into the subarachnoid spaces, but never into the subdural space.

The comparative anatomists gave relatively little attention to the connective-tissue spaces around the ear and there was consequently no great advance secured from this aspect of the problem. Hasse (1873, account taken from Retzius) investigated embryos of various mammals, but his results are confusing. Concerning the lymph tracts of the inner ear he showed that in man and other mammals, in embryonal and adult stages, there exists a channel to which he gave the name ductus perilymphaticus, which is the same channel through which the injectionists had forced their fluids into the scala tympani. Hasse described this as provided with a sac which connects on the one hand with the cavum subarachnoideale (the outer epicerebral space after splitting the brain membranes into pia and arachnoid) and into a lymph-vessel on the other. This drainage path of the "perilymph," according to him, is not the only and in fact is not the chief drainage path; a similar path, consisting of a funnel-shaped sheath of arachnoid, projects into the internal auditory meatus accompanying the acoustic and facial nerves. In a later paper, Hasse (1881) reverses the importance of these two channels and describes the perilymph as flowing chiefly through the ductus perilymphaticus into the peripheral lymph system in the region of the jugular foramen, the same channel also draining the cerebrospinal fluid of the subarachnoid cistern. There is also, according to him, some drainage from the subdural space through the internal auditory meatus.

Of more importance is the description of Retzius (1884). In his large monograph on the ear the gross and finer morphology of the periotic spaces and especially of the higher mammals, is described in greater detail and completeness than had previously been done. The comparative embryology of the spaces is referred to by Balfour (1885). He speaks of lymphatic spaces (p. 522) as forming in the mesoblast between the membranous labyrinth and the cartilage. These spaces are partially developed in *Sauropsida*, but become larger and more important in mammals, where they form the two scalæ and the space surrounding the utricle and semicircular ducts. According to him the scalæ begin to develop at the basal end of the cochlea, the cavity of each being gradually carried forward toward the apex of the cochlear canal by a "progressive absorption of the mesoblast."

The descriptions of Retzius (1884) and of Kölliker (1861 *b*, 1884) and also the chapter in the sixth edition of the "Gewebelehre," rewritten by v. Ebner (1902), have had a prevailing influence on the present conception of the character and development of the tissues of the otic capsule. There should be mentioned with

these also the work of Krause (1901), who studied the development of these structures in a number of vertebrate forms. He finds that the first traces of the formation of a "perilymphatic space" occur some little time before the formation of the scala tympani, in the region lateral to the utricle and saccule. At this point the perilymphatic tissue becomes gradually fluidified and there arises between the lateral wall of the two sacs and the cartilaginous wall of the labyrinth a large perilymphatic space—the cisterna perilymphatica—to which the foramen ovale serves as a direct approach. From this cistern the space-formation spreads into the cartilaginous semicircular canals and simultaneously there begins the formation of the scalæ, resulting finally in a cavity system that incloses the entire membranous labyrinth. Reference is made by him to the ductus perilymphaticus of Hasse, which connects this system with the subarachnoid spaces. This duct opens at one end in the vestibular part of the scala tympani and in the jugular fossa at the other. Concerning its development, nothing further was known.

In studying the development of the otic capsule, one is led into the general question of the growth of hyaline cartilage, for which there is an extensive literature and which is beyond the scope of the present paper. For general papers on this subject the reader is referred to those of Retterer (1900), Mall (1902), and Bardeen (1910). Other papers that may be mentioned as dealing particularly with the histogenesis of the skull are those of Solger (1889) and Filatoff (1906). An experimental study by Lewis (1907) should be referred to, in which it is shown that the production of the cartilaginous capsule is dependent upon the presence of the epithelial vesicle and that a transplanted otic plate becomes surrounded by cartilage derived from the tissue of the host.

The gross morphology of the cartilaginous capsule of the ear has been described for several embryonic stages in connection with the cartilaginous skull as a whole, and mention may be made in this connection of the work of Gaupp (1906) and Terry (1917) on certain vertebrate forms and the papers of Levi (1900) and Macklin (1914) on the human embryo. The writer has also had the opportunity of studying a reconstruction of the otic capsule in a human embryo 21 mm. long made by Professor W. H. Lewis and one of a 43-mm. embryo made by Dr. Macklin, both of which were modeled in this laboratory and have not yet been published.

MATERIAL AND METHODS.

The observations recorded in this paper are made on human embryos and cover the period included between 4 mm. and 130 mm., crown-rump length, which is approximately equivalent to the period between the fourth and the sixteenth week of fetal life. The embryos were taken from the collection made by Professor Mall and that now belongs to the Department of Embryology of the Carnegie Institution of Washington. With two exceptions they had already been prepared in serial sections. In most of the stages the whole embryo is included in the sections, in some of the older ones the head alone is included, and in the two specimens that were especially prepared for this investigation the sections include only the region of the temporal bone. In the following table are listed the embryos that were found

particularly suitable for the purpose at hand. They are arranged in the apparent order of development. The measurements given are those under which they are listed in the catalogue of the collection, and they all signify crown-rump measurement. Where sections were photographed this is indicated and the slide number, followed by the row and number of section, is given.

Table of Specimens.

Crown-rump length, millimeters.	Catalogue No.	Direction of section and thickness in microns.	Section photographed.	State of development of the periotic tissues.
4	588	Coronal... 15	6–6–7	Beginning condensation of periotic mesenchyme.
9	721	Tranverse. 15	5–2–1	Distinct condensation of periotic mesenchyme.
11.8	1121	Coronal... 40	6–3–4	
11	353	Coronal... 10	16–3–4	Definite capsules of mesenchyme surrounding labyrinth.
13	485	Coronal... 40	10–1–3	Membranous canals begin to separate off from main pouch.
12.5	317	Coronal... 20	.....	
13 5	695	Transverse. 10	.....	
16	406	Sagittal... 20	.....	Condensed mesenchyme becomes precartilage.
15	719	Transverse. 40	3–7–3	
14	144	Sagittal... 40	4–1–3	Differentiation of precartilage into permanent and temporary types.
16	409	Transverse. 20	6–3–5	
17 2	424	Transverse. 50	3–4–1	
17	296	Coronal... 20	.....	Distinct capsules forming around precartilage nuclei in some areas.
17	576	Sagittal... 15	.....	
21	460	Transverse. 40	14–1–1	
23	966	Coronal... 40	30–3	
23	453	Sagittal... 20	.....	
24	455	Transverse. 30	.....	
26.4	1008	Sagittal... 40	10–2–2	
26	1199	Coronal... 40	39–1–3	
27	756a	Coronal... 50	47–2	Beginning formation of definite periotic reticulum around canals.
27	875	Sagittal... 40	.....	Precartilage becomes true cartilage.
30	86	Coronal... 50	46–2	Reticular area surrounds epithelical canals.
33	145	Sagittal... 50	7–1–3	
33	211	Sagittal... 50	14–3	
35	199	Sagittal... 50	58–2	Beginning space-formation in vestibular region.
37	1318	Coronal... 100	42–1	
37	972	Sagittal... 50	20–1	Dedifferentiation of true cartilage into precartilage in region of canals.
39	362	Sagittal... 50	.....	
43	886	Coronal... 100	42–3	Rudimentary periotic cistern present and scala tympani can be recognized.
50	84	Transverse. 50	146–2	Scala vestibuli can be recognized.
46	95	Sagittal... 100	72–1	
50	224	Sagittal... 50	20–1	
50	184	Sagittal... 50	23–2	Rapid dedifferentiation of precartilage around canals into reticulum.
52	448	Sagittal... {25 / 100}	33–2	Vascular network established around canals.
50	96	Sagittal... 100	12–2	
59	267	Sagittal... 20	29–1–2	
73	1373	Transverse. 10	9–3–1	Perichondrium and membrana propria present in their early form.
80	172	Transverse. 100	191	
85	1400–30	Transverse. 100	43–2	
130	1018	Transverse. 50	30–1	Beginning formation of periotic spaces in region of canals.

In studying the development of the cartilaginous capsule and the histogenesis of the periotic reticulum it was found necessary to prepare enlarged photographs of the special regions studied. By having these all made on the same scale of

enlargement it was possible to follow from stage to stage the change in volume and in form of the constituent tissue masses. Some of the photographs are reproduced on Plates I and II. In drawing conclusions from such photographs account was taken of the fact that the technique of preparing the serial sections introduces an element of uncertainty in that some embryos in the process of embedding shrink more than others. This is particularly so in human embryos, where there is necessarily some difference in the freshness of the material at the time it is obtained. Furthermore, even in the same embryo some tissues are affected by the technique more than others. Due allowance was made for these factors.

In order to determine the form and relations of the periotic-tissue spaces, wax-plate models of the membranous labyrinth and the surrounding spaces were reconstructed after the Born method. Advantage was taken of the improvements in the method devised by Lewis (1915). The serial sections were photographed at a suitable enlargement on bromide paper. By means of a preliminary model of the membranous labyrinth the necessary reconstruction lines were established and transferred to the bromide prints. From these prints the membranous labyrinth and the periotic spaces were then traced on wax plates. After cutting out from the plates the areas corresponding to these structures the plates were piled and the resultant cavity was filled with plaster of paris. When the wax was finally melted off there remained a permanent plaster cast of the objects desired at a definite enlargement. Views of these models are shown on plate 4.

In outlining the periotic spaces it was found necessary to adopt an arbitrary rule as to how much should be included in the model. The smaller spaces of the reticulum surrounding the main cavities can be seen coalescing to form larger spaces, and these in turn coalesce with the main cavity as it advances into new territory. There is thus a considerable range in the size and completeness of the spaces in any one section. The main spaces and the larger adjacent ones that communicate with them are outlined by a membrane-like border. This characteristic was adopted as the guide in determining which spaces to admit into the model; only those possessing a more or less complete border of this kind were included.

DEVELOPMENT OF THE CARTILAGINOUS CAPSULE OF THE EAR.

When the present study was undertaken the writer's interest concerned more particularly the process of conversion of the periotic reticular tissue into the walled-off spaces that constitute the scala tympani, the scala vestibuli, and vestibular cistern. It was soon found, however, that this could not be satisfactorily treated without a consideration of the earlier history of this tissue and its relation to the surrounding cartilaginous capsule. Therefore, a preliminary survey was made of the earlier histogenetic processes of all the mesenchymal elements of the inner ear. The character of these processes will form the subject-matter of the first part of this paper. In brief, they include: (1) the original condensation of the mesenchyme around the otic vesicle; (2) the subsequent differentiation of the condensed mesenchyme into precartilage on the one hand and periotic reticular tissue on the other; (3) the differentiation of true cartilage and its manner of growth and alteration in

form. After considering these, we shall be prepared in the second part of this paper to take up the alterations in the periotic reticular tissue that lead to the formation of the periotic spaces.

CONDENSATION OF THE PERIOTIC MESENCHYME.

If one looks at the otic vesicle in a human embryo from 4 to 5 mm. long, just as the endolymphatic appendage is becoming constricted off from the remainder of the vesicle, it will be found that the mesodermal tissue surrounding it is about the same in its appearance as that in other regions. There is the brain-wall, the otic vesicle, the ganglion mass connecting them, a few blood-vessels, and the ectoderm; otherwise there is to be seen only a more or less uniform mesenchymal syncytium lying between these structures. Close against the vesicle the nuclei are perhaps a little more numerous. This can be seen in figure 5, which is taken from an embryo 4 mm. long (Carnegie Collection, No. 588). The section passes through the otic vesicle in its longest diameter and shows dorsally the endolymphatic appendage as it appears at this time. Lateral to the otic vesicle is the primary head-vein. A network of capillary vessels is spreading over the brain-wall, not extending quite to the ventral median line. Along the median margin of this sheet of capillaries there forms a larger channel which gradually separates itself from the capillaries and takes part in the formation of the basilar artery, as has been described in the chick and pig by Sabin (1917). The mesenchymatous tissue is denser in some regions than in others. The nuclei are quite sparse ventral to the brain-wall near the median line, becoming perceptibly more numerous as we approach the ear-vesicle. This increase in the number of nuclei in the neighborhood of the vesicle marks the beginning of the mesodermal condensation that is to form the otic capsule. It is not yet possible, however, to outline a definite layer of these nuclei.

When embryos are examined that are a little older than this it is found that a condensation of the mesoderm around the otic vesicle can be clearly recognized. Such a stage is shown in figure 6, which is from a photograph of a section of a human embryo 9 mm. long (Carnegie Collection, No. 721). Under low magnifications it is apparent that the mesoderm in the region of the vesicle is denser than the adjoining mesoderm, and particularly so on the lateral and ventral surfaces of the vesicle. The condensation of the mesoderm is also beginning on the median surface of the vesicle, but the process there is somewhat slower. The endolymphatic appendage, however, is free from any surrounding condensation; the mesoderm appears to be unaffected by its presence. The section in figure 6 passes transverse to the long axis of the vesicle. A small portion of the brain-wall is shown that is slightly retracted from the surrounding mesenchyme. The area of condensed tissue surrounding the vesicle is thick enough to extend from the surface of the vesicle to about half the distance from the vesicle to the ectoderm.

When analyzed under higher magnifications, it is found that the compact appearance around the vesicle is due to several factors. As compared with the mesenchymal syncytium of the adjoining parts, the nuclei here are slightly larger, are more numerous, and are closer together. The intervening protoplasmic syn-

cytium is also denser and possesses wider trabeculæ, with correspondingly smaller spaces between them. This condensed tissue abuts, on the one hand, directly against the epithelial wall of the vesicle and forms a limiting membrane, as can be seen in places where the epithelium is retracted through shrinkage changes. On the other hand, it is directly continuous with the general mesenchymal syncytium, the transition between the two, however, being quite abrupt, as can be seen on careful scrutiny.

In embryos between 11 and 13 mm. long, which is just before the first semicircular duct is separated off from the main labyrinth by the apposition and absorption of the intervening labyrinthine wall, the condensation of the mesoderm has advanced in thickness and extent so that it forms a nearly complete capsule for the epithelial labyrinth. Such a stage is shown in figure 7, which is taken from a human embryo 11 mm. long (Carnegie Collection, No. 353). This capsule encasing the labyrinth is thicker and denser on the lateral and ventral surfaces of the labyrinth, including the ventral pouch that is to form the cochlea. It remains incomplete on the median surface in the region of the nerve terminations. This latter space is occupied by the rootlets of the acoustic nerve-complex which bridge the short distance between labyrinth and brain and which are invested by a rich plexus of blood-vessels. It is this area that eventually becomes the internal auditory meatus. Slightly more caudal, near the glossopharyngeal nerve, can also be made out a deficient portion of the capsule that corresponds to the fenestra cochleæ (rotunda) and the aquæductus cochleæ. A third opening through the capsule is brought about by the endolymphatic appendage. This does not become encased by the capsule, but emerges dorsally to lie between the brain membranes and the skull. At first this latter opening is one in common with the internal auditory meatus. It very soon becomes separated off by the growth of the condensed tissue around the neck of the endolymphatic appendage. In figure 7 the section passes through the long axis of the membranous labyrinth. Only the vestibular portion is shown with the endolymphatic appendage opening out of it. The section passes transverse to the thickened margins of the pouches that are to form the superior and lateral semicircular ducts.

Thus at this time there is completely formed a condensed area of embryonic connective tissue surrounding the labyrinth that corresponds closely in form to the cartilaginous capsule into which it is about to be converted. On examining it under higher magnification there is found very little, aside from the condensation, that distinguishes it as yet from ordinary embryonic connective tissue. The condensed appearance is due to several factors. In the first place, the nuclei are more numerous in a given area. They also tend to be larger and rounder. Furthermore, the protoplasmic syncytium between the nuclei is denser, consisting of more numerous and more branched trabeculæ. In an embryo 16 mm. long, which had been stained with iron hematoxylin and erythrosin (Carnegie Collection, No. 406) the trabeculæ between the nuclei appear granular. This appearance is due to the presence of minute nodes that are found along the trabeculæ and which are stained deeply by the erythrosin, and add to the density of the tissue. Similar nodes are

found in the same embryo in the ordinary mesenchyme in that neighborhood, but are less numerous. This condensed tissue differs in one respect quite definitely from ordinary mesenchyme, in that it is almost devoid of blood-vessels, excepting along its margins. To all appearances it abuts, as in younger specimens, directly against the epithelial wall of the labyrinth.

DIFFERENTIATION OF PRECARTILAGE.

The histogenetic changes which mark the beginning of the conversion of the condensed mesenchyme into a cartilage-like tissue make their first appearance just after the separation of the semicircular ducts from the main vestibular pouch. This occurs in embryos about 14 mm. long. In embryos about 30 mm. long the otic capsule has the appearance and gives the tinctorial reactions of true cartilage. Thus, in embryos between 14 mm. and 30 mm. long, the otic capsule consists of a tissue that is intermediate between a condensed embryonic connective tissue and cartilage, and this intermediate form is known as precartilage.

The appearance of the otic capsule just at the time the canals are forming is shown in figure 8, which is from an embryo 15 mm. long (Carnegie Collection, No. 719). The section passes horizontally through the labyrinth. A portion of the utricle is shown at the bottom of the photograph, and detached from it, above, is the superior semicircular duct. A streak extending from the duct to the utricle still persists. This streak represents the wall of the labyrinth that formerly occupied this place and is now absorbed close up to the inner margin of the duct. Surrounding the capsule is a plexus of blood-vessels.

On examination under higher magnifications it is found that the tissue forming the capsule at this time differs very little from the condensed mesenchyme which we have seen in the younger stages. The most noticeable difference is that the nuclei are beginning to stand more apart from each other. This can be seen by comparing figures 7 and 8. In the former the section is 10μ thick, in the latter the section is 40μ thick. In spite of being four times thicker, the section of the older specimen shows only about the same number of nuclei that are seen in the thinner and younger specimen in figure 7.

Between the nuclei there are numerous branching slender processes. The spaces between the processes are not as clear as the spaces in the adjoining subcutaneous connective tissue, but contain a homogeneous substance that stains very slightly with such a dye as alum cochineal. The accumulation of this substance is doubtless related to the spreading apart of the nuclei and to the alteration in the branching processes that begins to show at this time. In certain regions the processes between the nuclei become less branched. Larger ones become more prominent and the smaller ones begin to disappear. A common arrangement is to find two or more larger processes uniting to form a loop at the side or at one or both ends of the nucleus. This feature is characteristic of precartilage. There is very little tendency as yet to an accumulation of denser protoplasm around the nuclei.

In embryos about 16 and 17 mm. long the optic capsule takes on a definite precartilaginous character. This stage is shown in figure 9, which is from a photograph of an embryo between 17 and 18 mm. long (Carnegie Collection, No. 144). The embryo is listed in the collection as 14 mm. long, which is its measurement on the slide. Instead of this we use here its estimated formalin measurement, so as to conform to the other embryos, whose measurements are all given as in formalin. The section passes sagittally through the labyrinth. Above is shown the posterior semicircular duct and just below the center is shown the caudal end of the lateral semicircular duct, at the point where it widens out to join the utricle. By this time the differentiation of the tissue has advanced far enough so that one can properly speak of an otic capsule that is readily distinguished from any other condensed connective tissue. The outlines of the capsule are everywhere distinct. It fuses in part with the cartilaginous skull and it is continuous with the stapes. Embedded in it is the epithelial labyrinth together with its ganglionated nerves. The capsule envelops them entirely, except at the nerve entrance which is to form the internal auditory meatus, also at a point in the region of the jugular fossa that is to become the fenestra cochleæ and at the opening through which the endolymphatic appendage emerges.

On comparing figure 9 with figure 8 it will be seen that in addition to an actual increase in size the otic capsule is less uniform in appearance at this older stage. There are areas of denser tissue, or, rather, areas of more deeply staining tissue, which extend as streaks through the capsule inclosing other areas of less deeply staining tissue. The areas of less deeply staining tissue are in the immediate neighborhood of the semicircular ducts, completely encircling them and abutting directly against the epithelial wall of the ducts, as in the previous stage.

On examination under high magnification we find that the tissue forming the otic capsule at this time (embryos 17 mm. long) has for the greater part been transformed into precartilage. Precartilage, as seen in fixed material that has been sectioned and stained by the usual methods, differs from condensed mesenchyme chiefly in the alteration in the network of branching processes that extend between the nuclei. In condensed mesenchyme these appear as a syncytium of delicate refractile processes. In precartilage some of these become more sharply marked and linear, and are looped together so as to inclose an irregular space near each nucleus; the others become very finely subdivided and eventually disappear. While these latter processes are disappearing the area in which they lie takes on a homogeneous appearance. It does not take the stain, but it is more opaque than the inclosed spaces around the nuclei. Thus, instead of a syncytium the precartilage tissue gives the appearance of cell-islands separated from each other by a homogeneous matrix.

Regarding the exact structure of this slightly opaque substance our material does not suffice to warrant an opinion. This question must be approached by special methods. I may add, however, that remnants of fibrillar processes are found embedded in this substance for some little time after the walling-off of the encapsulated spaces or cell-islands. Each cell-island consists of a nucleus encap-

sulated by a clear space that varies in size and shape and whose contour seems to be formed by the persisting processes of the original syncytium. At first the nucleus is accompanied by very little condensed protoplasm, but this gradually accumulates after the formation of the encapsulated spaces and constitutes a cell-body of endoplasm. The nuclei continue to divide after the encapsulation and they can be seen in all stages of the process. The space shares in the subdivision and for a time each daughter nucleus inherits its own share of the space. The encapsulated spaces, in an embryo 17 mm. long (Carnegie Collection, No. 576), which had been stained deeply with hematoxylin and eosin, contained a homogeneous substance that was tinged with eosin. The substance was collected around the nucleus and filled more than half of the space of the capsule; but clearly it was not protoplasm and was not to be confused with the endoplasmic cell-body which forms later. None of this substance was found in the matirx surrounding the capsules.

The embryos in the Carnegie Collection that, on account of the stain that was used and the thinness of the sections, show particularly well the process of the differentiation of the encapsulated spaces are as follows: No. 576, 17 mm.; No. 409, 16 mm.; No. 296, 17 mm.; No. 409, 18 mm.; No. 455, 24 mm.; and No. 453, 23 mm. The order in which they are given indicates their relative development. In all of them areas are found showing different stages in the differentiation. On comparing them one could come equally well to two different conclusions regarding the encapsulation of the nuclei and the differentiation of the matrix. One could either say that the mesenchymal syncytium during the precartilage period undergoes a fusion into a semi-solid, homogeneous, slightly opaque mass in which the fibrils disappear and which forms the precartilaginous matrix, while at the same time selected spaces of the original syncytium develop a sharp margin and become encapsulated, each containing its own nucleus, or, one could say that the substance composing the matrix is deposited in the meshes of the syncytium, replacing most of the fibrils and obliterating the spaces except those selected ones that are inclosed by persistent processes and are encapsulated with an adjoining nucleus. One can not, however, see much evidence for considering the encapsulated spaces as of vacuole formation. They are certainly not vacuoles of the endoplasm, for the endoplasm does not make its appearance until after the spaces have taken on their characteristic form.

DIFFERENTIATION OF CARTILAGE.

The transition from precartilage to cartilage is a gradual differentiation that takes place in the otic capsule of embryos between 25 and 30 mm. long. If one examines an embryo 30 mm. long, such as shown in figure 11, it will be seen on comparing it with younger stages that the main capsular mass has undergone a distinct maturation. This transition is marked by a considerable increase in the amount of matrix combined with a more complete encapsulation of the nuclei, or cartilage cells as we may now call them. As the matrix increases in amount it also changes in its chemical composition, so that it is now possible to stain it differentially.

This tinctorial reaction makes an arbitrary point at which it may be said that precartilage becomes cartilage.

All parts of the capsule do not take part in this process equally. It has already been mentioned that during the period of differentiation of the precartilage the tissue of the otic capsule loses its homogeneous character and some areas of it begin to appear more dense than others. Immediately surrounding the semicircular ducts is quite a wide area of precartilage that appears less dense, which in turn is inclosed by the main precartilaginous mass of the capsule whose nuclei give it the appearance of greater density. This can be seen very well in figure 10. When we come to embryos between 26 and 30 mm. long this contrast between the two varieties of precartilage becomes more sharply defined, though the relative compactness of the arrangement of the nuclei becomes reversed. The semicircular ducts are then everywhere encircled by an area of temporary precartilage that differs from the rest of the capsule and which is not to become true cartilage, but is to be hollowed out to form the cartilaginous canals. This process of hollowing out the cartilaginous spaces and replacing with reticular connective tissue the precartilage that originally filled them forms a very interesting feature in the development of the otic capsule, to which we will refer later.

The difference between temporary precartilage and true cartilage is shown clearly in figure 11. This section passes transversely through the lateral semicircular canal of an embryo 30 mm. long (Carnegie Collection, No. 86). An area of temporary precartilage surrounds the epithelial duct, forming a dark circular field outside of which is the more permanent capsular mass. Examination under higher powers shows that the temporary precartilage differs from the main mass in that the nuclei are arranged somewhat concentrically, and there is less space between them than exists in the latter, which is the reason for its darker appearance. Furthermore, whereas the temporary precartilage around the semicircular ducts retains the general histological features that were seen in the younger stages, the main capsular mass has matured into well-defined cartilage. A specimen of about this same age is shown in figure 13 (Carnegie Collection, No. 199, 35 mm. long). This specimen was stained only in hematoxylin, which emphasizes the matrix. In such a preparation the cartilaginous matrix is stained intensely blue, whereas the temporary precartilage around the semicircular ducts takes the stain only in its nuclei. The reverse picture is shown in figure 12, where the tissues show an intense nuclear stain. This is taken from an embryo of about the same age as that shown in figure 13. Here, on account of the nuclei and the intervening dense protoplasm, the temporary precartilage forms a dark mass around the semicircular duct. Figures 12 and 13 are like a positive and negative and approximately indicate the outlines of the eventual cartilaginous canal. The area of temporary precartilage gradually retracts towards the border of the more permanent cartilage, as we shall see in the later stages, and as it does so the space becomes occupied by a reticulum of connective tissue.

In passing from embryos 30 mm. long to older stages, such as shown in figures 12, 13, and 14, the tissues show some advance in the degree of their maturation.

Their intense stain-reaction causes the area of temporary precartilage to stand out very conspicuously. On examining under higher powers the section shown in figure 12 (Carnegie Collection, No. 972, 37 mm. long), it is seen that the nuclei in the precartilage area are somewhat more numerous and are more compactly arranged than in the same area in figure 11. The darker appearance as contrasted with the surrounding cartilage is also due partly to the fact that the compact mass of internuclear protoplasm is distinctly tinged by the acid stains, whereas in the surrounding permanent cartilage the matrix is nearly devoid of any color, having been decolorized by the differential stain.

In addition to the staining reaction there is now a marked difference in structure between the more permanent cartilage and the temporary precartilage. The latter retains its precartilaginous character. Its more peripheral cells show a slight tendency to capsule-formation. A common form among these is an oblong nucleus with thickened elongated processes at the four corners, resembling the pronged egg-case of the shark, the spaces between the processes on each side of the nucleus being parts of the incomplete capsular space. These cells are arranged in circular lines parallel with the circumference of the canal. The transition into true cartilage is rather abrupt, and on advancing into this region one meets with a characteristic matrix, embedded in which are the completely encapsulated nuclei. The temporary precartilage in its more central layers, near the reticulum, does not show any tendency towards encapsulation. Its nuclei are arranged in concentric layers with a small amount of compact protoplasm between them, resembling an early stage of fibrous connective tissue.

A layer of blood-vessels marks the junction of the temporary precartilage with the reticulum surrounding the semicircular duct. This reticulum appears lighter than the surrounding precartilage because of the free spaces between its slender trabeculæ. Furthermore, the nuclei are not quite so numerous and are more irregularly arranged. The reticulum does not advance very rapidly in its development, and it is not until we come to embryos between 40 and 50 mm. long that we meet with an extensive reticulum. The development of this reticulum will be described after we have taken up some of the subsequent changes in the cartilage.

GROWTH AND ALTERATION OF FORM OF THE CARTILAGINOUS CANALS.

In embryos 30 mm. long the cartilaginous labyrinth has attained approximately the adult form. Its subsequent development is primarily an increase in size to accommodate the growing membranous labyrinth. If a cast of the superior cartilaginous canal of an 80 mm. embryo be compared with a similar cast of the same canal in a 30 mm. embryo, it will be seen that the general form of the canal in the older specimen is much the same as in the younger specimen But its diameter and length have both increased, the diameter being nearly doubled and the length trebled; furthermore, its linear curvature corresponds to an arc with a considerably longer radius. In reality, therefore, the developing cartilaginous labyrinth is continually undergoing changes, both in size and form. The histological evidence of these changes constitutes one of the most interesting features in the development of

this region, and although it does not directly concern the development of the contained perioticular spaces, yet it may not be out of place to point out some of the elements of this process as they are seen in our material. In fact, the cartilaginous capsule of the ear is an especially favorable place for studying the general question of growth of cartilage, for two reasons: (1) there are, on account of the intricacy of form of the labyrinth, many kinds of cartilaginous changes found there that are necessary to accommodate its growth, including the deposit of new tissue and the removal of old tissues; (2) the topography is so well marked by known landmarks that all of these changes as well as the location and direction of growth can be easily followed.

Growth of cartilage is usually considered to be of two kinds, which are distinguished from each other by being either interstitial or perichondrial. Interstitial growth is described as consisting of an increase in the amount of hyaline matrix and the growth and proliferation of the encapsulated cartilage cells. The new cells form new capsules to a certain extent, but a point is finally reached beyond which the newly proliferated cells continue to occupy their parent capsule . From this variety of growth there results a uniform intumescence of the tissue without producing any marked change in its form. This manner of growth forms a large element in the increase in size of some parts of the capsule of the ear. In those parts, however, where a change in form is involved the growth is more like that described under perichondrial growth and consists of a new deposit of cartilage along the borders of the older cartilage, the consituent cells passing through a precartilage stage. In the otic capsule this latter type of growth is actively going on even before a definite perichondrium is established. The deposit of new cartilage along the margin of older cartilage and the removal of old cartilage by dedifferentiation are indeed the main factors in the process through which the form of the ear-capsule is modeled.

The excavation of established cartilage can be studied by comparing sections through the semicircular canals at different stages, such as appear in figures 11, 12, 14, and 15. These are all sections through the same canal (lateral), taken in about the same position, and are enlarged the same number of diameters. It is, of course, possible that they were shrunken in different degrees in the process of embedding; this discrepancy, however, is probably not enough to interfere with their showing the approximate increase in size of the cartilaginous canal at the respective ages. A crude measurement of the perimeters of the canals as seen in the original photographs (100 diameters) yields the following circumferences: 30-mm. embryo, 115 mm. circumference; 37-mm. embryo, 132 mm. circumference; 43-mm. embryo, 152 mm. circumference; 50-mm. embryo, 192 mm. circumference. It is evident that we are dealing with an enlarging space and that a study of its receding edge must give the histological picture of the replacement of true cartilage by other tissue, either by dedifferentiation or by direct metaplasia.

If, with this process in mind, one makes an examination of the specimen shown in figure 11 (Carnegie Collection, No. 86) it will be seen under higher magnification that a rather definite border can be made out separating the general mass of true

cartilage from the inner zone of temporary precartilage surrounding the semicircular ducts. The true cartilage has developed a considerable amount of matrix separating the encapsulated nuclei or cartilage cells. The margins of the capsules stand out as sharp refractive lines. The matrix lying between the capsules is slightly opaque and is beginning to take a differential stain. A narrow intermediate or transition zone separates the true cartilage from the precartilage; this zone is characterized by the presence of flattened and partially collapsed capsules between which there is very little or no matrix. The refractive margins of these overlapping, incomplete capsules give the appearance of wavy lines that run parallel with the margin of the canal. The same appearance is not seen in other regions of the otic capsule in younger stages, where precartilage is differentiating into cartilage.

In the process of cartilage differentiation in most parts of the otic capsule there is considerable intercapsular material at the time the margins of the capsules become conspicuous. The capsules are separated by the matrix-forming syncytium. Thus, there are not the conspicuous wavy lines due to overlapping capsules, such as characterize the intermediate zone. The transition between this zone and the true cartilage on one hand and the temporary precartilage on the other is quite abrupt in both instances. On entering the zone of precartilage there is found between the nuclei, instead of the wavy refractive capsular lines, a framework having more the character of a granular syncytium, with only here and there the suggestion of a beginning capsule. This, it will be remembered, is a condition the true cartilage exhibited in its earlier period. It is the intermediate zone to which we should address our especial attention, and it is this zone that moves outward as the cartilaginous canal widens.

Text-figure 1 shows a section through these zones in a fetus of about the same age as the one just described. This section is taken through the lateral canal of a fetus 33 mm. crown-rump length (Carnegie Collection, No. 145). The intermediate zone stands out con-

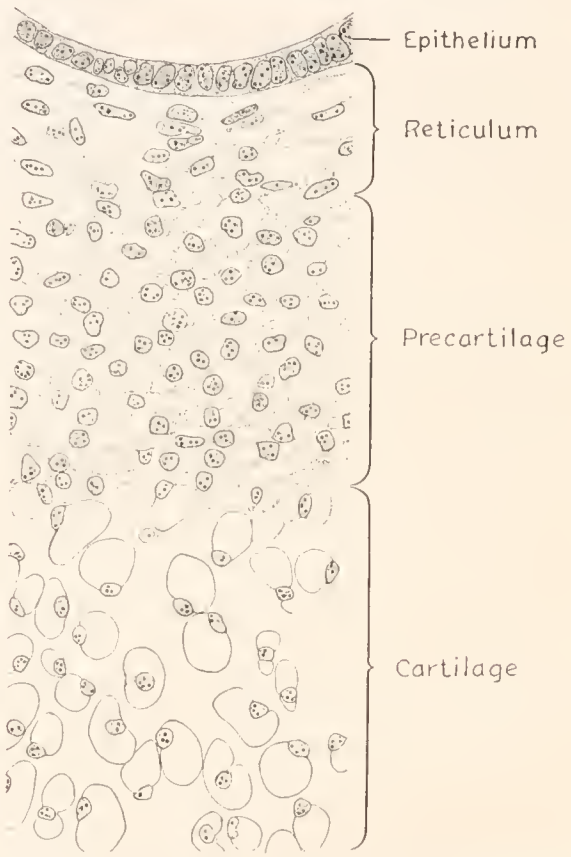

FIG. 1.—Detail of a section through the lateral semicircular canal in a human fetus 33 mm. long (Carnegie Collection, No. 145, slide 7, row 1, section 3). The section is 50μ thick and is enlarged 376 diameters. It shows the epithelial wall of the semicircular duct and the tissue zones that intervene between it and the cartilaginous capsule. In the outer part of the pre-cartilage zone is an intermediate area which is a transitional form between cartilage and precartilage. It is characterized by the scant amount of matrix and the incomplete and flattened condition of the capsule.

spicuously at the junction of the cartilage with the precartilage. Its wavy refractive lines are so compact that under low powers the whole zone appears as a dark rim outlining the cartilaginous margin of the canal. The compactness of these lines varies in different embryos of about the same age and even varies in the two borders of a given canal.

This latter condition can be seen in figure 14, which represents the lateral semicircular canal of a fetus 43 mm. crown-rump length (Carnegie Collection, No. 886). It will be noted that the peripheral two-thirds of the intermediate zone (toward the right hand) forms a dark, heavy margin between the true cartilage and the encircled precartilage, whereas the central one-third (toward the left hand) is wider and much less distinct. It can also be seen that the place at which this intermediate zone is well marked corresponds to the direction of the excavation necessary to allow for the growth of the canal and to make room for the elongating semicircular ducts of the contained membranous labyrinth. In this case the expansion must be toward the periphery of the cartilaginous capsule, *i.e.*, toward the right side of the photograph. From studying various fetuses it seems to be true that where excavation of cartilage is actively going on at such a place there is found a prominent intermediate zone along the inner margin of the cartilage. Sometimes the line is uniform around the entire rim, but usually it is more marked on one side of the canal than on the other, and in such cases it is always toward the direction of the excavation of the cartilage, as can be judged from the topography of the labyrinth.

If an older specimen is examined, such as the one represented in figure 15, the character and relative position of the cartilage and precartilage are found to be the same as in the 30-mm. stage just described. They have, however, undergone an alteration to allow for the enlargement of the cartilaginous canal. Figure 15 shows the lateral canal in a human fetus about 50 mm. crown-rump length (Carnegie Collection, No. 95). The fetus is catalogued as being 46 mm. long, but this is apparently the slide-measurement. In its development it corresponds to fetuses 50 mm. long, formalin measurement, and this measurement is used so that it will accord with the other fetuses. Since figures 11 and 15 represent sections through the same canal taken at about the same place and under the same enlargement, one can superimpose them, one upon the other, and thus determine the change that has occurred between the two stages. If this is done it will be seen that the area that was precartilage in the 30-mm. stage is replaced by reticulum in the 50-mm. stage. There is just as much or more precartilage in the latter, but it has moved outward into the area that was previously true cartilage. In other words, the enlargement of the cartilaginous canal has been obtained by a process of excavation based on the dedifferentiation of true cartilage into precartilage and the latter in turn into reticulum. This is shown under higher magnifications in text-figures 2 and 3, which show sections of these same canals under the same enlargement and placed side by side for the purpose of better comparison. It can be seen in these two figures how the cartilage of 30-mm. stage becomes dedifferentiated into the precartilage of the 50-mm. stage and the border along which this process is in active operation forms the intermediate zone, which is characterized by its wavy, refractile

lines. The precartilage in turn is gradually dedifferentiated into the periotic reticulum. In this way the margin of the true cartilage gradually recedes from the epithelial duct, and the last of the precartilage is eventually dedifferentiated into a reticulum.

Along with the process of excavation of cartilage there must go the laying-down of new cartilage. For instance, as the lateral cartilaginous canal enlarges it also moves laterally, so that the distance between it and the carti-

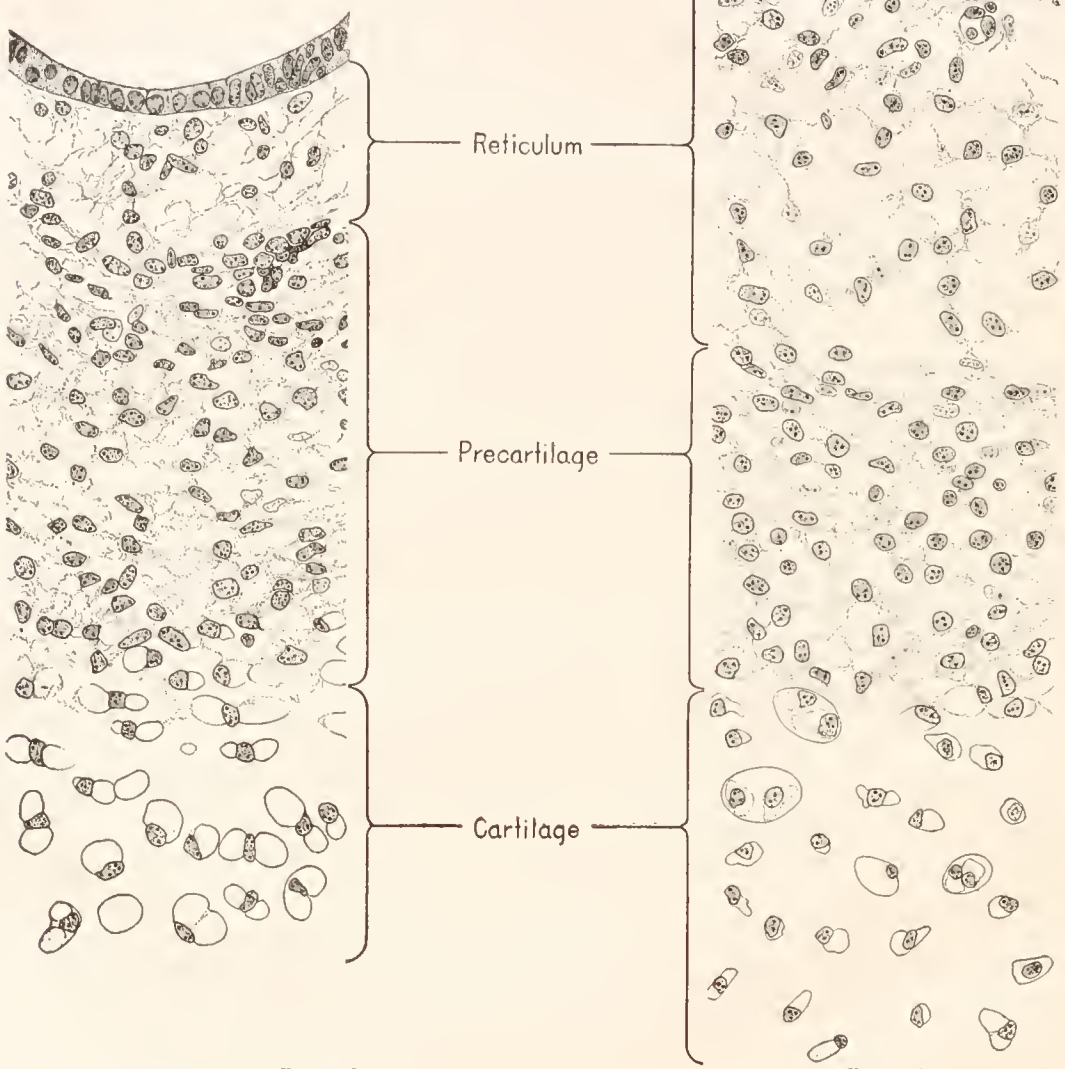

Figure 2. Figure 3.

Fig. 2.—Detail of the lateral semicircular duct in a human fetus 30 mm. long (Carnegie Collection, No. 86), being the same as that shown in figure 11. The section is 50μ thick and is enlarged 470 diameters. It shows the epithelial wall of the duct and the character of the surrounding tissues that lie between it and the cartilage.

Fig. 3.—Detail of the lateral canal in a human fetus 46 mm. long (Carnegie Collection, No. 95, slide 72, section 1). This is the same canal as that shown in figure 15. The section is 100μ thick and is enlarged 470 diameters. By comparing it with figure 2 one can see how the cartilaginous canal is enlarged by the dedifferentiation of cartilage into precartilage and the precartilage in turn into the periotic reticulum.

laginous vestibule increases, producing relatively a lateral migration of the space as a whole. Such a migration involves the excavation of the established cartilage on its lateral margin and the formation of new cartilage on its median margin. On its lateral margin true cartilage is being dedifferentiated into precartilage and on its median margin precartilage is differentiating into cartilage. It is this phenomenon that determines the conditions shown in figure 14. On the right can be seen the prominent intermediate zone, indicating an active excavation of cartilage, and on the left the line of transition between cartilage and precartilage presents the same picture as that seen in the stage of differentiation of the latter into the former. One is forced to conclude that the cartilaginous tissue of the otic capsule is capable of differentiation and dedifferentiation in its earlier stages, at least up to the time of the completion of the encapsulation of the cartilage cells. This progressive and retrogressive adaptability of the cartilaginous tissue makes possible the changes that are necessary in the growth and alteration in form of the labyrinth.

DEVELOPMENT OF PERIOTIC RETICULAR CONNECTIVE TISSUE.

The formation of the connective-tissue reticulum surrounding the semicircular ducts is first indicated by a cluster of darkly stained nuclei that lie along the central edge of the ducts in embryos soon after the ducts are formed and before the differentiation of the cartilage is completed. In figure 9 such a cluster is seen just under the posterior duct in the upper part of the photograph. In figure 10, which shows the lateral semicircular duct of an embryo 27 mm. long (Carnegie Collection, No. 756*a*), a similar cluster of nuclei can be seen just under the duct, in reality just median to it. These foci mark the points at which the formation of the reticulum begins. It is not, however, until we come to embryos about 30 mm. long that we find a definite reticulum. At that time, as is shown in figure 11, a narrow lighter area can be made out, situated between the epithelial wall of the duct and the temporary precartilage. It is the development of this area at the expense of the temporary precartilage that results in the reticulum in which the periotic spaces are subsequently formed. This area consists of a mesenchymal syncytium containing irregularly shaped clear tissue spaces and is characterized by the presence of numerous blood-vessels and connecting capillaries. The larger vessels are found resting against the inner margin of the temporary precartilage. They sometimes indent it, but never penetrate it to any extent. Such vessels can be seen in figures 11 and 12. The presence of these blood-vessels is coincident with the appearance of the reticular tissue.

In describing younger stages the statement has been made that the temporary precartilage abuts directly against the epithelial wall of the semicircular duct. This statement is based only on the gross appearance. On careful scrutiny of the tissue that immediately surrounds the ducts in embryos between 14 mm. and 20 mm. long a few mesenchymal cells can be found which possibly do not belong to the temporary precartilage. These cells may very well represent some of the indifferent mesenchyme, and possibly also some angioblasts. It is conceivable that these surround the otic vesicle in its earliest stages and are inclosed along with

the otic vesicle by the condensed tissue of the otic capsule, where they remain in contact with the epithelial labyrinth in a resting condition until the embryo approaches 20 mm. in length. They then show activity and by the time the embryo is 30 mm. long we find them converted into a vascularized reticulum which forms a definite area surrounding each semicircular duct and completely separating it from the receding precartilage. The area of reticulum advances as the precartilage becomes hollowed out. This can be seen by comparing figures 11, 12, 14, 15, and 16, all of which are reproduced on the same scale of enlargement.

From the histological appearance one could maintain that the reticulum is derived from a few predestined mesenchymatous cells which, after a latent period, undergo proliferation and occupy the space that is vacated by the receding precartilage in the manner described above, the growth of the reticulum perhaps being the cause of the recession of the cartilage. But one could equally well maintain that the reticulum is derived entirely from the precartilage; that it is not a predetermined tissue, but simply precartilage that has undergone dedifferentiation. It is entirely possible that the isolated cells included with the epithelial labyrinth are angioblasts only, everything else being indifferent mesenchyme. In the early stages, where only a few cells are concerned, this matter can not be determined, the histological difference between early precartilage and other embryonic cells not being sufficiently great for their certain recognition. In the later stages, however, it is quite evident that precartilage tissue is actually converted into a reticulum; that the replacement of the temporary precartilage by a reticular connective tissue is accomplished by a process of dedifferentiation, or direct metaplasia, just as we have previously seen in the case of the dedifferentiation of cartilage into precartilage.

In this connection it is instructive to compare again figures 11 and 15, and also figures 2 and 3, which are details of the same under higher magnification. They show under the same enlargement a section through the lateral canal made in about the same position and cut at the same thickness. It will be noticed that the space occupied by precartilage in the younger stage is entirely filled in by reticulum in the older stage. There is in the older stage, however, more precartilage than before, but it now occupies a more peripheral position. With the change in the position of the precartilage area there is a corresponding enlargement of the lumen of the true cartilage, *i. e.*, the cartilaginous canal. It is clear that we are dealing here with a dedifferentiation of true cartilage into precartilage on the one hand and a dedifferentiation of precartilage into reticulum on the other. These factors, as we already have seen, are of great importance in the alteration in form and size of the cartilaginous canals.

In younger stages, as in figure 10, the epithelial semicircular duct lies near the center of the area of temporary precartilage. When the reticulum develops it makes its first appearance, and its growth continues more marked along the concave side of the duct than on the convex side—that is, on the side toward the utricle rather than toward the periphery of the capsule. On this account the epithelial duct loses its central position and gradually comes to lie along the peripheral border of the cartilaginous canal, where it eventually becomes attached to the periosteum.

This eccentric position gives the canal the largest arc that is possible in the space in which it lies. It marks the point of thrust of the elongating duct against the cartilaginous chamber that confines it and it is in this direction that the cartilage must be excavated to make room for the further growth of the duct.

The spread of the reticulum into the surrounding precartilage is rather slow at first. There is very little advance made in fetuses between 30 mm. and 43 mm. long, as can be seen by comparing figures 11 to 14. In figure 14 the reticulum can be recognized as a crescentic-shaped area on the central side (toward the left) and partially surrounding the epithelial duct. In the figure it is about 0.8 cm. wide at its widest point. The surrounding precartilage is also of about the same width, but it is uniformly wide around the whole circumference of the cartilaginous canal. In fetuses about 50 mm. long the dedifferentiation of precartilage into reticulum makes more rapid progress. The change is quite abrupt at this time. Figures 14, 15, 16, and 17 form a series in which is shown the alteration from a small amount of reticulum to an almost complete reticularization of the cartilaginous canal. These changes are found in fetuses varying from 43 mm. to 52 mm. long. In comparing these figures one would expect that the membranous duct would be found progressively larger in the series of photographs if they were correctly arranged in the order of their age. But it should be remembered that the tissues show different degrees of response to the fixing reagents. This is particularly so in respect to the epithelial duct; in figures 14 and 17 it is distended, as can be seen by its thin wall, while in 15 and 16 it is contracted. The order in which they are arranged corresponds to their relative age, as far as could be determined by the records of the fetuses and general appearances of the sections.

In figure 15 there is a zone of precartilage, about 0.8 cm. wide in the photograph, which in reality is true cartilage that has been dedifferentiated into precartilage. The reticulum extends from the inner border of this to the membranous duct. In figure 16, which is a section through the posterior canal of a fetus 50 mm. long (Carnegie Collection, No. 184), the dedifferentiation of precartilage into reticulum has occurred faster than that of cartilage into precartilage. There is practically none of the latter to be seen; the whole of the space between the margin on the cartilage and membranous semicircular duct is filled in by reticulum. Along the central margin of the duct there are still seen thick clusters of proliferating nuclei which are associated in part with the development of the blood-vessels and in part with the modification of the reticulum that takes place around the wall of the membranous duct.

It has been noted that precartilage is free of blood-vessels, whereas the reticulum is vascularized from the very first. Part of the dedifferentiation of precartilage into reticulum consists of the invasion of blood-vessels into the precartilage region. In the early stages of the reticulum the larger vessels hug closely against the precartilage and continue to do so as the latter recedes from the epithelial duct, as can be seen in figures 11, 12, and 14. Later, with the abrupt dedifferentiation of the remaining precartilage into reticulum, the larger vessels do not follow the receding margin of the cartilaginous canal, but form vascular arches that are

suspended in the reticulum, as can be seen in figures 15, 16, and 17, and from these a network of small vessels branches toward the membranous duct on the one hand and the cartilaginous wall on the other.

In figure 17, which is a section through the posterior semicircular canal in a fetus 52 mm. crown-rump length (Carnegie Collection, No. 96), the reticulum is more mature in its appearance than any that have thus far been described. There is practically no precartilage to be seen. The reticulum now only lacks the membrane-like thickening of its inner and outer margins to render it complete. At the inner margin the cells arrange themselves into a fibrous coat that constitutes the membrana propria of the membranous duct. At the outer margin is formed the perichondrium, the development of which will now be considered.

DEVELOPMENT OF THE PERICHONDRIUM.

In the description of the development of the periotic reticulum we have seen how it begins as a small focus along the central border of the epithelial semicircular duct and spreads at the expense of the temporary precartilage, forming as it does so a crescentic-shaped area of reticulum inclosing the duct. We have also seen how the invasion or spread of the reticulum into the surrounding area of precartilage is brought about, at least in the later stages, by a dedifferentiation of the latter into the former.

Furthermore, along with this latter process, the inner margin of cartilage surrounding the duct is dedifferentiated into precartilage, so that a new area of precartilage becomes established as the old area disappears. The conversion of precartilage into reticulum in the later stages, however, is more rapid than the conversion of cartilage into precartilage, and consequently there comes a time when the precartilage has nearly all disappeared. In such specimens the reticulum extends practically from the epithelial duct to the margin of the cartilaginous canal. The qualifying term "practically" is used because the inner and outer margins of the reticulum are modified in a special manner. The inner margin becomes condensed into a membrane-like coat of fibrous tissue that constitutes the membrana propria of the membranous canal. The outer margin at about this time undergoes changes that result in the formation of the perichondrium.

In discussing the perichondrium it is important to keep in mind the active alterations in the tissue along the margin of the cartilage that accompany the growth of the labyrinth. It has been seen how the enlargement of the cartilaginous canals and their alterations in form and position is obtained partly by excavation of cartilage and partly by the laying down of new cartilage, the excavation being accomplished by its dedifferentiation into precartilage and reticulum, and the new cartilage being built up through a precartilage stage from the periotic reticular tissue. Throughout the entire period of growth of the cartilaginous canals the elements of this continual transformation exist along their margin. The margin during this period is in a state of temporary equilibrium and is capable of advancing or receding as the conditions determine.

The first and relatively the major part of the hollowing-out of the cartilaginous canals is complete before the perichondrium makes its appearance. This is illustrated, for instance, by the fetus of 52 mm. crown-rump length, in figure 17, where there is as yet no indication of it shown. In fetuses between 40 and 50 mm. long the zone of precartilage surrounding the margins of the canals, as seen in figures 14 and 15, might be mistaken for perichondrium. This area, however, in fetuses slightly older is converted almost entirely into reticulum. Kölliker (1879), in the second edition of his text-book on embryology, pictures a transverse section through the lateral canal of a rabbit embryo (fig. 457, page 735), in which this zone of precartilage is labeled as periosteum of the future bone.

The real perichondrium does not make its appearance until the fetus reaches a a length of about 70 mm. A specimen of this age is represented in text-figure 4, which shows a segment of the posterior semicircular canal in a fetus 73 mm. crown-rump length (Carnegie Collection, No. 1373). On examination of this specimen it is found that there is a distinct condensation of the reticulum along its inner margin, so that it forms a membrana propria for the epithelial duct with which it is in contact. This area has largely lost its reticular character and now resembles embryonic fibrous connective tissue. Along the outer margin of the reticulum a similar condensation of its trabeculæ has taken place, forming a thin fibrous lamina or membrane near the margin of the cartilage. This is the perichondrium in its early form. It does not abut directly against the cartilage, but is separated from it by a thin layer of transition tissue that is in process of dedifferentiation from precartilage into reticulum.

Passing inward from the cartilage, the transitions are rapid from cartilage to precartilage, from precartilage to the tissue that is in transition to the reticulum and then to the perichondrium. These are found as narrow zones that merge quickly from one into the other. One should remember that the cartilaginous canal has not reached its full size yet, and that the margin of the canal is still in an unstable condition. However, as the canal becomes larger and the tissues more mature, it is found that the transitions between the different zones become more abrupt and in this process the precartilage zone becomes relatively much narrower. This can be seen by comparing text-figures 3 and 4. The width of the reticulum in these two figures can not be compared, because they represent different canals, lateral and posterior, and no attempt was made to take them from the same relative positions. The fact that the reticulum is narrower in figure 4 has no significance in the question of growth. The wide precartilage zone in figure 3 as compared with that in figure 4, on the contrary, has a direct bearing on the relative age of the two specimens. A relatively wide zone of precartilage is characteristic of younger stages. After fetuses become 70 mm. long the precartilage zone becomes quite narrow, so that the transition from cartilage to perichondrium is relatively abrupt. In older specimens one might easily obtain the impression that the perichondrium rested directly against the cartilage, as doubtless it does in the adult. In the oldest fetus examined, 130 mm. crown-rump length, there is still found a distinct though

narrow precartilage-reticular transitional zone between the cartilage and the perichondrium. Presumably this indicates that the margin is still in an unstable condition.

After the perichondrium has made its first appearance it rapidly becomes thicker and more conspicuous. In a fetus 80 mm. crown-rump length (Carnegie Collection, No. 172) it is found as quite a dense fibrous coat, more than twice as thick as that shown in the 73 mm. embryo in figure 4. It is clearly separated from the cartilage and precartilage by a narrow zone of reticular tissue.

The character of the perichondrium as existing in slightly older fetuses is shown in figure 18, which represents a section through the posterior semicircular canal of a fetus 85 mm. crown-rump length (Carnegie Collection, No. 1400–30). Here the perichondrium consists of a relatively broad zone of embryonic fibrous connective tissue, which in the photograph is about 5 mm. wide, encircling the whole canal. It can be seen on the median side (to the left) that it is separated from the cartilage and adjacent transforming precartilage zone by a narrow, lighter area, which under higher magnification is found to consist of reticular tissue. The membrana propria at the inner margin of the reticulum is fairly well developed and it can be seen how it forms a supporting coat to the epithelial duct.

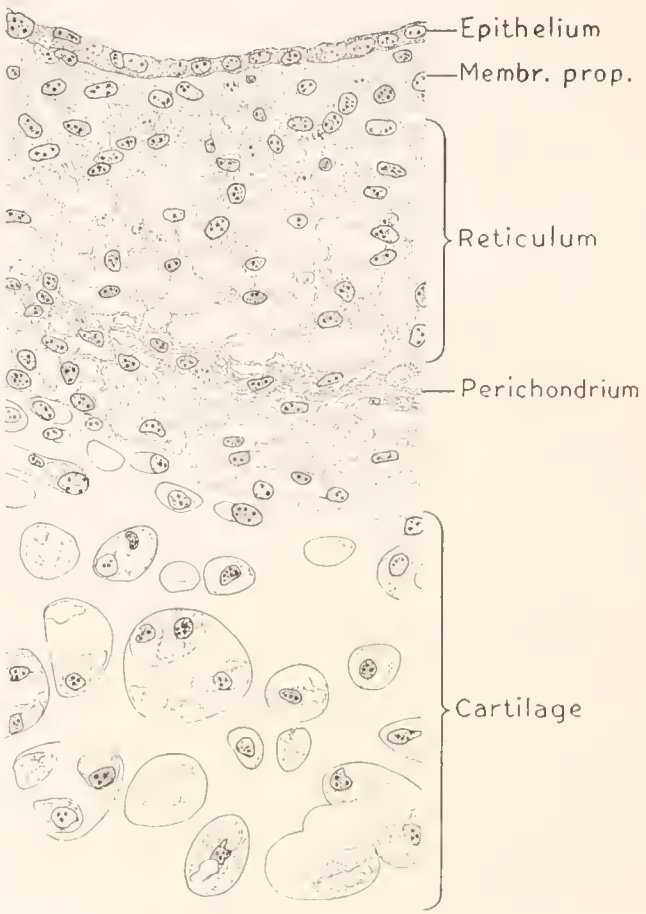

FIG. 4.—Detail of the posterior canal in a human fetus 73 mm. long (Carnegie Collection, No. 1373, slide 9, row 3, section 1). The section is 10μ thick and is enlarged 376 diameters. It shows how the inner margin of the reticulum becomes condensed into the membrana propria of the epithelial duct and the outer margin into the perichondrium. The perichondrium does not lie in direct contact with the cartilage, but is separated by a narrow zone of tissue which consists of precartilage, into which the cartilage is still being dedifferentiated.

When one examines the cartilaginous semicircular canals in fetuses 130 mm. long there can no longer be any question as to the identity of the perichondrium. A specimen showing the superior semicircular canal at this stage is represented in figure 19, which is taken from a fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018). The blood-vessels are injected with India ink. The main cartilaginous mass in this specimen is quite mature; the capsules are well defined and the cartilage cells now possess a considerable amount of granular body-protoplasm.

In many instances capsules are found containing more than one cartilage cell, showing the tendency to cell columns.

A casual glance at a section under lower powers might indicate that the inner margin of the cartilage is in direct contact with the perichondrium. Examination under higher magnification, however, shows that between the thick perichondrium and the cartilage there is a narrow zone of dedifferentiated cartilage. In it the matrix has largely disappeared and the capsules have collapsed and are flattened out, allowing the elongated endoplasm of adjacent cartilage cells to come in contact, separated only by the remnants of the capsular margins. Dyes that stain endoplasm red cause this zone to appear as a deep-red line. This zone represents a state of transition between cartilage and precartilage and its presence doubtless indicates that the margin of the cartilage is still in an unstable condition. The narrowness of the zone and the abruptness of the transition are characteristic of later stages, where the process is more gradual and relatively small in amount. The transition from this zone to the perichondrium is likewise abrupt. The perichondrium consists of a dense protoplasmic stratum thickly studded with nuclei, and has all the appearance of late embryonic fibrous connective tissue. It is of about the same thickness around the whole margin of the canal. At the outer margin (toward the right) it fuses with the membrana propria of the epithelial duct, thereby forming an attachment which is regarded as a suspensory ligament for the support of the membranous labyrinth. The trabeculæ of the reticulum extending between the membrana propria and the perichondrium are just beginning to break apart, allowing the adjacent spaces of the reticulum, as they are seen in section, to coalesce in the formation of larger spaces.

Having completed the review of the early history of the reticulum and its formative relations to the adjacent tissues, we are now in a position to consider the development and the fate of these larger spaces in the reticulum, which have hitherto been generally known by the misleading term "perilymphatic spaces."

DEVELOPMENT OF PERIOTIC TISSUE—SPACES.

In the preceding pages of this article the main features of the development of the cartilaginous capsule that incloses the membranous labyrinth have been described. We have traced the process step by step from the first condensation of the mesenchyme around the otic vesicle, through its differentiation into a precartilaginous mass and the maturation of the latter into true cartilage, with the formation through dedifferentiation of cartilaginous chambers in which the membranous labyrinth is suspended. It has been shown how these spaces within the cartilaginous capsule are modified in adaptation to the continued growth of the membranous labyrinth and how they finally come to be filled with an open-meshed reticulum which everywhere bridges the space existing between the membranous labyrinth and the surrounding cartilage. It has further been shown that the membrana propria supporting the epithelial part of the labyrinth on the one hand and the perichondrium on the other are derived from and serve as the limiting membranes of this reticulum. It is a modification in the meshes of this same reticulum

that results in the formation of the so-called perilymphatic spaces, or periotic spaces as they will be referred to in this paper, the development of which will now be outlined.

Thus far attention has been directed primarily to regions included in typical transverse sections through the semicircular canals. This was done for the purpose of uniformity and simplicity and because of the ease with which successive stages could be compared with one another. For studying the periotic spaces, however, the region of the canals is not so favorable, because the spaces are late in developing there, and even in their completed form they are not so well defined and highly differentiated as those in the region of the vestibule and cochlea.

The earliest evidence of a periotic space makes its appearance opposite the stapes. It is developed in the reticulum that fills the interval situated between the saccule, utricle, and the cartilaginous stapes. Even before the general periotic reticulum becomes very extensive, in embryos between 30 and 40 mm. long, it can be seen that its meshes are more irregular and more open in this region than elsewhere. This is the rudimentary form of the periotic vestibular cistern, which is the first space to become established.

DEVELOPMENT OF THE PERIOTIC CISTERN OF THE VESTIBULE.

Aside from the scala vestibuli and the scala tympani, the largest of the periotic spaces is the large reservoir situated between the tympanic wall of the bony vestibule with its articulated stapes and the vestibular chambers of the membranous labyrinth. This is the spatium perilymphaticum vestibuli (BNA) or the cisterna perilymphatica (Retzius). In order to eliminate the word lymphatic from the terminology it will be designated here as the cisterna periotica vestibuli, or less formally the periotic cistern. In this manner the descriptive term introduced by Retzius is retained.

Before there is any trace of the scalæ the initial steps in the formation of the cistern can be seen. This is well illustrated in an embryo 35 mm. long (Carnegie Collection, No. 199). This particular embryo is cut in a sagittal series and the sections on slides 53 and 54 show the periotic cistern in its most rudimentary form. It consists of an area of reticulum bounded by the utricle, saccule, ductus reuniens, the proximal end of the cochlear duct, and the ampulla of the posterior semicircular duct. The greater part of the periotic reticulum at this time (35-mm embryo) is characterized by a narrow and uniform mesh that is interrupted only by numerous capillaries branching through it; in the area mentioned, however, the spaces are larger and are more irregular both in shape and in size. They present the appearance seen along the semicircular ducts in considerably older embryos, for instance, in the 52-mm. embryo, as is shown in figure 17. From the very first the increase in the size of the mesh seems to be attained by the detachment and retraction of its constituent protoplasmic bridges, thereby allowing adjacent spaces to unite in the formation of composite large spaces. Thus in the above section a few irregular protoplasmic free-ends are seen still projecting into the newly enlarged spaces. This interesting histogenetic process will be taken up again later in connection with

the development of the two scalæ. The area of this rudimentary periotic cistern is as yet very small and merges indefinitely into the adjoining reticulum. It is not until we come to fetuses about 40 mm. long that it develops spaces of any considerable size, and it is not until we come to fetuses about 50 mm. long that we find a single large space with walls that are definitely outlined, so that it can be satisfactorily modeled.

In a fetus 43 mm. long (Carnegie Collection, No. 886), which is cut in a coronal series, the spaces forming the rudimentary cistern stand out much more definitely than is the case in the 35-mm. embryo that has just been referred to. There is now just opposite the stapes one space which is much larger than the adjoining spaces. On part of its margin the protoplasmic bridges are stretched along so as to form a smoothly curved continuous boundary, which is defective in some portions, and at such places the space merges with the adjoining secondary spaces. Within the space are some faintly refractive branching threads of coagulated plasma. The scala vestibuli is not yet laid down and the scala tympani is only represented by a moderate widening of the meshes of the reticulum in the neighborhood of the fenestra cochleæ (rotunda), along the basal border of the first turn of the cochlear duct.

In fetuses 50 mm. long the outlines of the cistern become very distinct, due to the marked increase in the size of its main cavity and to the more definite membrane at its junction with the rest of the reticulum. Its form and relations are shown in figures 26 and 27. They represent a median and a lateral view of a wax-plate reconstruction of this region in a human fetus 50 mm. long (Carnegie Collection, No. 84). Only the main cavity is shown in the model. At certain places around its borders the meshes of the reticulum are uniting into larger spaces and these in turn are taken up by the main cavity as it advances into the new territory. These smaller incomplete spaces were omitted in constructing the plates of the model. The rule was adopted that only the spaces that were outlined by a membrane-like border should be traced on the plates and included in the model. This rule was adhered to in all the models of this series.

Figures 26 and 27 show that the periotic cistern in 50-mm. embryos consists of a flattened, rounded, bursa-like cavity intervening between the stapes and the lateral surface of the saccule and adjoining utricle. It extends forward to the ampulla of the lateral canal and upward to the beginning of the crus commune. Posteriorly it crowds backward against the ductus reuniens, filling in the space between the utricle, saccule, and the proximal end of the cochlear duct. Both on its median and lateral surfaces there is no further opportunity for expansion except as the vestibule itself enlarges. The delicate membrane-like wall of the cistern hugs closely against the parts of the membranous labyrinth on the one side and the tympanic wall of the cartilaginous vestibule on the other, being separated from them only by a thin layer of the original reticulum. Along the dorsal margin of the cistern, however, there is room for expansion, and the reticulum in this region shows enlarging spaces in the process of uniting with the main cavity. On its ventral margin, near the cochlea and extending along the apical surface of the latter, there

is a definite row of reticular spaces actively coalescing and constituting the beginning of the scala vestibuli. These are shown in figure 21, which is a section of a fetus of about the same age. The spaces of the scala vestibuli lie between the cochlear duct and the cistern. This section also shows very well the relation of the stapes to the cistern. The scala tympani is already well started at this time, but its development is quite independent of the cistern. Within the cistern can be seen scattered clumps of faintly refractive granular threads of what seems to be a coagulated constituent of the plasma.

The subsequent growth of the cistern is shown in figures 28 to 31. Figures 28 and 29 show respectively a median and lateral view of a wax-plate reconstruction of the membranous labyrinth and its periotic spaces in a human fetus 85 mm. long (Carnegie Collection, No. 1400–30). The growth of the cistern here has kept pace with the increase in size of the labyrinth and maintains the same general relations as regards the stapes and the parts of the membranous labyrinth. The view of the cistern in figure 28 is an oblique one which would tend to mislead one as to its width. In reality it is relatively a little wider. It has also extended upward on the dorsal surface of the utricle and is beginning to creep along the inner side of the posterior end of the lateral semicircular duct. Ventrally it communicates freely with the scala vestibuli, which now extends well down along the cochlear duct.

The oldest stage studied is shown in figures 30 and 31. These show two views of a wax-plate reconstruction of these structures in a human fetus 130 mm. long (Carnegie Collection, No. 1018). At this time the periotic cistern has spread over the vestibular part of the membranous labyrinth, covering it nearly everywhere excepting at the macular portions where the nerves terminate. In figure 31 it can be seen that the mesial surface of the saccule is not covered; this lies close against the wall of the cartilaginous vestibule. The uppermost division of the cistern, situated between the crus commune and the ampulla of the posterior semicircular duct, does not yet open into the general cavity. It has formed separately and owing to the position in which it lies its coalescence with the other parts of the cistern is retarded; otherwise, free communication exists between all divisions of the cistern.

DEVELOPMENT OF THE PERIOTIC SPACES OF THE SEMICIRCULAR DUCTS.

From the descriptions given of the adult the reticulum along the ducts never develops a single continuous wide periotic space like that of the cistern and the two scalæ. There always remain a few trabeculæ, such as are seen in the cistern and scalæ in their earlier stages, and these constitute partitions which traverse the space and give it in sections the appearance of a series of separate spaces extending along the inner margins of the semicircular ducts. Although these spaces along the ducts are incomplete as compared with the cistern and scalæ, they are, however, entirely analogous with them in their formation.

The space along the lateral semicircular duct is the largest. Its posterior end exists as a continuation of the cistern. This can be seen in the lateral view of the model shown in figure 30, where the cistern extends for a considerable distance

along the inner border of the lateral duct. Along the other two ducts of the same specimen (130 mm. crown-rump length) the reticulum has commenced the process of space-formation, but complete channels are not yet established. A typical section through one of the semicircular ducts in a fetus of this size, and this is the oldest fetus studied, is shown in figure 23. As compared with the scalæ in the same fetus, as shown in figure 20, the space-formation along the ducts is very much retarded.

DEVELOPMENT OF SCALA TYMPANI AND SCALA VESTIBULI.

The scala vestibuli may be regarded as an extension of the cistern downward into the region of the cochlea and as such its growth starts from a focus opposite the fenestra vestibuli (ovalis). The scala tympani in a similar way makes its first appearance opposite the fenestra cochleæ. From these two foci the scalæ extend gradually downward along the cochlear duct as two separate spaces which do not communicate with each other until they reach the tip of the duct, where there is finally developed a free opening between them known as the helicotrema.

In their formation they go through a series of histogenetic changes essentially in the same manner that has been followed in the case of the formation of the cistern; this (as we shall see) consists of the enlargement of the spaces of the periotic reticulum that originally occupied this region, the enlargement being a result of the disappearance of the protoplasmic bridges of the reticulum, whereby adjacent spaces unite in the formation of composite larger spaces. This process continues until there is a single continuous space extending down along the cochlear duct representing each scala and at the margins of each of them there is developed a membranous arrangement of the reticular cells which completely walls off the space from the surrounding tissue. In these alterations in the reticular mesh and in the formation of the surrounding membrane there is an active change in the form of the reticular cells, which repeatedly adapt themselves to the new conditions. There is no evidence to indicate that any other cells take part in the formation of the scalæ.

The first evidence of the formation of scalæ is found in fetuses about 40 mm. long, which stage is a little later than the first appearance of the cistern. In a fetus 43 mm. crown-rump length (Cargnegie Collection, No. 886), along the proximal part of the cochlear duct on its basal surface there is a distinct widening of the meshes of the periotic reticulum. This is the beginning of the scala tympani. On the opposite side of the cochlear duct, where one would look for the scala vestibuli, the periotic reticulum retains its primitive appearance characterized by a narrow and rather uniform mesh. Thus the scala tympani makes its appearance slightly in advance of the scala vestibuli—that is, if we regard the latter as distinct from the cistern.

In fetuses 50 mm. long both the scala tympani and the scala vestibuli can be plainly identified, although they are still very incomplete. A wax-plate reconstruction of them, representing their form and their relation to the membranous labyrinth in a human fetus 50 mm. crown-rump length (Carnegie Collection, No. 84), is shown in figures 26 and 27, being a median and a lateral view respectively.

In preparing this and the models shown in figures 28 to 31, it is to be remembered that only those periotic spaces are included that were outlined by a membrane-like margin. In the adjacent reticulum there are spaces that are actively coalescing and gradually uniting with the main cavity. No attempt, however, was made to show such spaces in the models. From figures 26 and 27 it will be seen that the scala tympani is larger and more advanced in its development than the scala vestibuli. The latter is in its earliest stage and consists of hardly more than a row of enlarged reticular spaces which extend downward from the cistern along the dorsal and apical surface of the cochlear duct. A section through the scala vestibuli in another fetus of about the same age (Carnegie Collection, No. 448) is roughly shown in figure 21, the spaces of the scala being situated between the cistern and the cochlear duct.

The scala tympani consists of an elongated oval space lying along the basal surface of the proximal part of the cochlear duct, about corresponding to the proximal half of the first turn of the duct. In the main part it is a single space with a distinct margin separating it from the general periotic reticulum. In the more apical portion it tapers off into multiple incompletely united smaller spaces which actively coalesce as the process advances into the new territory along the duct. It is of interest to note that the most mature and the largest part of this scala, representing the focus at which it first appeared, is opposite the fenestra cochleæ (rotunda), just as the cistern forms opposite the stapes and the fenestra vestibuli. The scala tympani always begins at the same place and extends downward along the cochlear duct, at first a little in advance of the scala vestibuli, but subsequently the latter catches up with it and the two reach the tip of the duct at about the same time.

It is well known that the proximal portions of the cochlear duct mature sooner than the distal portions. One might expect that the accompanying periotic spaces would correspond in their development to the maturity of the duct and therefore the proximal parts of the scalæ would differentiate first. In other words, the maturation of the cochlea proceeds as a wave from the proximal end to its tip, involving all of its constituent structures as it passes along, including mesenchymal parts as well as epithelial.

This conception might explain the direction of development of the scalæ, but can hardly be applied to the cistern, the vestibular representative of the scala vestibuli. One can not say that those portions of the membranous labyrinth lying opposite the focus of development of the cistern (that is, the lateral walls of the saccule and utricle) mature in advance of the rest of the labyrinth. There is no indication that a wave of differentiation passes through the epithelial elements of the labyrinth in the same direction and synchronously with the extension of the cistern as it advances from its primary focus upon the roof of the utricle and over on its median surface. In the case of the cistern it seems much more likely that the point at which it first appears is determined by the position of the stapes, which is doubtless an expression of the physical relation that subsequently exists between the two. By analogy this would yield additional significance to the relation existing between the fenestra cochleæ and the point of beginning development of the scala tympani.

In dealing with the cistern and also with the scalæ one should not consider them as insignificant accessories that merely fill in the waste intervals between the membranous labyrinth and the surrounding cartilage. From studying their development it becomes apparent that they have a morphological individuality in many respects as definite as that of the ossicles themselves. They make their appearance at a definite time and at definite places, they spread in a definite manner, and eventually they attain a form and structure that are adapted to a definite function. This becomes more and more evident as we examine older stages.

The form and relations of the scalæ in fetuses between 12 and 13 weeks old are shown in figures 28 and 29. These figures show median and lateral views of a wax-plate reconstruction of the membranous labyrinth and the surrounding periotic spaces in a human fetus 85 mm. crown-rump length (Carnegie Collection, No. 1400–30). Attention has already been directed to these figures in the description previously given of the cistern. The scala vestibuli can be seen in figure 28. Above, it opens freely into the cistern and extends downward along the apical side of the duct as a single main space, possessing a rather uniform diameter. It extends along the first two turns of the duct, gradually tapering off and showing a less mature character in its distal portions. Along the second turn of the duct the spaces are incompletely fused and the contour becomes correspondingly irregular. As a rule the peripheral margin of the scala is less mature and more irregular than the central margin. The scala vestibuli does not connect with the scala tympani at any point as yet. The two are separated in the first place by the cochlear duct and then more centrally by a framework of connective tissue in which are the radiating bundles of the cochlear nerve with the nodes of ganglion cells that form the spinal ganglion. These latter structures are not shown in the model; they occupy, however, the V-shaped groove seen between the two scalæ.

The scala tympani, as can be seen in figure 29, extends downward on the basal side of the cochlear duct along its first two turns. This corresponds to about the same linear dimension as that of the scala vestibuli. In its proximal portion it shows a greater area in cross-section than the latter, but further toward the apical region it is of about the same size and in some places it is even smaller. The peripheral margin of the scala tympani is distinctly more irregular than the central margin. The irregularity is due to spaces along this margin that are actively coalescing with the main space, but in which the fusion is not yet complete. The irregularity of this margin is thus an indication of the direction of the expansion of the scala. As the diameter of the whole cochlear mass increases, it is evident that the main growth of the scala must radiate outward in a peripheral direction. This is accomplished by the continual assimilation of new reticular spaces along this margin. At the proximal end of the scala tympani can be seen an oval depression which corresponds to the fenestra cochleæ (rotunda) and with which it stands in intimate relation.

In fetuses about 16 weeks old the form and relations of the scalæ have nearly attained the adult conditions, and this represents the oldest stage studied in connection with the present paper. The conditions found at this time are shown

in figures 30 and 31, which present median and lateral views of a wax-plate model of a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018). On comparing the scala tympani and scala vestibuli as seen in these figures with those in figures 28 and 29, it will be seen that they are larger in cross-section and more nearly cover the cochlear duct. Furthermore, they now extend to the extreme tip of the duct and communicate with each other across its central margin, thus forming a helicotrema. A section through this point can be seen in figure 25, in which these structures are shown as seen under low magnification. It will be noted that now, even as far as the tip of the cochlea, each of the scalæ consists of a continuous principal space, though both are more mature and larger in their proximal portions. Along the first turn of the cochlear duct they are walled off by a smooth membranous margin which separates them from the adjacent reticular tissue. The spaces of the latter do not seem to be taking any further part in the process of enlargement of the scalæ. Along the second turn of the cochlear duct, a section of which is shown in figure 20, the coalescence of reticular spaces with each other and with the scalæ is still in active operation. This produces a greater irregularity of the scalæ than is shown in the model. The subsidiary spaces are shown as a solid mass; the slender clefts separating them are not represented. The nearer one approaches the tip of the duct the more immature are the scalæ, until the condition is reached that is shown in figure 25, where the membrane-like margin is quite incomplete and the spaces merge irregularly with the surrounding reticulum. Thus a single specimen, if studied in its different parts, shows several stages in this interesting process of the formation and growth of the scalæ.

The figures grouped on plate 3 illustrate some of the histological features of this process. An early stage in space-formation is shown in figure 23. This is a section through the canal region where the changes in the reticulum are late in making their appearance. In fact, the periotic spaces never reach the same degree of differentiation here that occurs in the case of the cistern and scalæ. The initial steps, however, are the same, and this figure presents very well the appearance of the periotic reticulum as it begins to open up into larger spaces. Unmodified reticulum is characterized by a rather uniform narrow mesh. The essential change in space-formation consists in the disappearance of some of the trabeculæ of the mesh, with the consequent coalescence of the corresponding adjacent spaces. The trabeculæ consist of the protoplasmic processes of the constituent cells of the reticulum and their disappearance is to be explained in either of two ways: It is possible that owing to some property of the fluid element of the tissue the protoplasmic strands are dissolved or liquefied; this would account for their complete disappearance. On the other hand, the same result could be accomplished by an alteration in the form of the cell processes. A given trabecula could separate at either end, or at some point along its line, and the free ends of protoplasm could then retract and reshape themselves and become a part of the remaining framework. Whether we are dealing with a liquefaction of tissue or with active motility of the cell, protoplasm involving detachment and retraction of the trabeculæ can

not be definitely determined by observations of fixed tissue; but the appearance of sections where the process is in active operation seems to the writer to indicate the latter.

In the above paragraph and elsewhere in this paper reference is made to trabeculæ serving as "partitions" between "spaces" and the disappearance of trabeculæ resulting in the "coalescence of adjacent spaces." In making this use of the term "space" it should be explained that it is done in a descriptive sense, in application to the appearance of the tissue as seen in sections in which form human embryological material is mainly available. In thin sections of a reticular tissue one sees trabeculæ as partitions separating adjacent spaces. The same tissue in a mass would show that the spaces everywhere communicate freely with each other, like the spaces in a sponge, and that the trabeculæ are thread-like strands which at the best are very incomplete partitions. Instead of a meshwork containing many small spaces, one could perhaps equally well describe reticular tissue as a single large space traversed by many trabeculæ. If the latter practice were adopted, one would describe the development of the tissue-spaces with which we are concerned as a process of gradual decrease in the number of traversing trabeculæ, with the result that the mesh thereby becomes coarser. For descriptive purposes, however, it is convenient to refer to the intervals between the strands of the mesh as spaces, at the same time not granting them the significance that is attached to such membrane-lined tissue-spaces as are represented by the vestibular cistern and the two scalæ, though the latter are in reality derived from them.

In figure 23 the free detached ends of the trabeculæ will be noted everywhere, as is characteristic of this stage of development. It is a necessary step in the coalescence of adjacent spaces. The detached trabeculæ seem to be gradually retracting and adapting themselves to the formation of larger spaces. Their constituent protoplasm reshapes itself as a smooth border or as a part of other trabeculæ. Larger spaces necessitate longer trabeculæ, and as trabeculæ become longer they also tend to become heavier. These phenomena are all in evidence in the spreading and enlargement of the scalæ.

Figure 20 shows a characteristic view of the scalæ as seen under low magnification. It will be noted that the scala vestibuli is relatively mature at this point; the scala tympani, however, is in the act of spreading peripherally, so as to underlie, as it eventually will do, the future basilar membrane. The scala tympani finally reaches the peripheral margin of the cochlear duct, and it does this by the coalescence of the enlarging reticular spaces, which become incorporated with the main cavity of the scala. This can be observed better in figure 22, which shows a detail of the same section as seen under higher magnification. By comparing this figure with figure 20 the exact location can be readily made out. A portion of the main cavity of the scala is indicated and to the right of this are a few enlarged reticular spaces that are uniting with each other and will in the end become part of the main space. In addition to the enlarged reticular spaces there is a certain amount of residual undifferentiated reticulum. It is this tissue that will play the

part of an adventitial coat to the completed scala. The trabeculæ that separate the enlarged spaces seem to be under tension and about ready to snap apart. In fact, in most sections one can see the fragmentary ends of trabeculæ where this interruption of continuity has apparently occurred.

The differentiation of the margin of the scalæ constitutes the final feature in their maturation. During the period in which the enlargement of an individual scala is being brought about by the coalescence of enlarging reticular spaces, the margins of the main cavity can be seen to consist of smooth, delicate strands of nucleated protoplasm that resembles in all essentials that of the trabeculæ between the large reticular spaces. These linear margins are interrupted here and there by openings into adjacent spaces, but they tend to form a continuous line that definitely marks off the space from the adjacent reticulum. An early stage in the formation of such a margin is shown in figure 25, where the margin is indicated at a few places, but for the most part the space abuts against the surrounding ragged reticulum. The margin of the space is more complete in the scala tympani shown in figure 22, but it is still thin and delicate and can be easily opened up to allow the taking in of new spaces. If we examine the borders of more mature spaces we find them inclosed by a firmer membrane, which finally reaches a state that will probably not admit of any further opening up for the coalescence of additional spaces. Any further growth must thereafter be limited to simple distention of the wall of the space with the consequent adjustment of its constituent cells. Such a condition is represented in figure 24. This shows a more mature section of the wall of the scala vestibuli, being a detail of the same section shown in figure 20. The only difference between such a membrane, as we must now call it, and the corresponding structure in younger stages is its density; it is wider and its protoplasm perhaps more opaque, or in other words, more protoplasm is accumulated there.

If figures 24, 22, 25, and 23 are compared and followed in that order, it will be seen that the lining membrane of the scalæ can be traced backward, step by step, to the ordinary trabeculæ of the periotic reticulum. There is no histological evidence that any new cells enter into its formation. It seems to be simply a product of the proliferation and adaptive reshaping of the cells already there. In its final form the margin of the space resembles an endothelial membrane. One could describe, as immediately lining the space, a thin membrane with flattened nuclei, which is supported underneath by a thin coat of nucleated protoplasm that has the form of fibrous connective tissue. The former, judging only from its final appearance, one might designate as endothelium and thus make a distinction between it and the underlying tissue. In its histogenesis, however, it differs in no way from the rest of the wall and the difference that exists later seems to be merely the result of its adaptation to the existing physical conditions. Its early behavior is entirely different from that of vascular endothelium. Thus if its final appearance is stressed and the term endothelium is used for its designation, it must be done with a considerable amount of reservation. It is preeminently a place where the term mesothelium could be used with great advantage.

COMMUNICATION OF PERIOTIC SPACES WITH ARACHNOID SPACES.

The relation of the scala tympani and scala vestibuli to the subarachnoid spaces surrounding the hind-brain is of considerable interest, both on account of the possibility of their functional relationship and on account of the similarity that exists in their development. For a satisfactory investigation of the establishment and the character of the communications that are formed between these two allied systems of tissue-spaces, one should resort to other methods than those used in the present study, and, furthermore, one should examine older fetuses than those described here. In fact, a problem lies here that would be well worth careful study.

Certain observations, however, were made in the course of the above investigation that bear relation to these matters, and they will be briefly outlined here. In the first place, the histological picture of the periotic reticulum is essentially the same as that of the early stages of the pia-arachnoidal tissue investing the central nervous system. The enlargement of the meshes of the latter and the formation of the subarachnoid spaces and the arachnoid cistern, as has been recently described by Weed (1917), correspond exactly with the appearance seen in the histogenesis of the periotic spaces in the ear. The periotic spaces are not, however, extensions of the arachnoid spaces that have invaded the cavity of the cartilaginous labyrinth. If this were so we should find them first appearing among the rootlets of the vestibular and cochlear nerves, along which the subarachnoid space extends for some little distance. Instead, they begin at points where there can be no connection with the arachnoid tissue and their direction of growth is quite independent of it. The periotic spaces may be analogous to the arachnoid spaces, but they are not identical with them, nor are they an extension of them.

According to the descriptions of the adult anatomy of the ear, a communication becomes established between the scala tympani and the subarachnoid space near the fenestra cochleæ, the so-called aquæductus cochleæ. Vague and conflicting statements are also made concerning a communication through the internal auditory meatus connecting the arachnoid spaces with the scalæ. Such communication must be established quite late. In the oldest fetus examined, 130 mm. crown-rump length, they did not yet exist. As to the latter communication, it can be seen that the arachnoid spaces extend peripherally through the internal auditory meatus along the trunk of the acoustic nerve-complex, and slender pockets and clefts from them extend along the larger bundles of the cochlear nerve; they terminate, however, before reaching the margins of the scalæ, and there is no evidence at this stage that there is ever to be a communication between them and the scalæ. As to the aquæductus cochleæ, in the 130 mm. fetus it can be plainly seen that it is already forming as a derivative of the arachnoid spaces, although the communication with the scala tympani is not yet established. The arachnoid spaces invest the glossopharyngeal nerve and extend down along its trunk and pass directly by the region of the fenestra cochleæ (rotunda). A thin-walled tubular pouch projects from these spaces, leaving the nerve trunk and extending obliquely toward the scala tympani in a direction that would meet it just distal to the fenestral

impression on its basal surface. This fundament of the aquæductus cochleæ is present in fetuses 85 mm. crown-rump length, but is longer in the 130 mm. fetus, where it nearly reaches the scala tympani. The communication must be established soon after this.

SUMMARY.

The changes in size and form which the cartilaginous capsule of the ear undergoes during its development in the human embryo are accomplished in part by a progressive and in part by a retrogressive differentiation of its constituent tissues. Throughout the entire period of growth, as far as material was available for study, it was found that the margins of the cartilaginous cavities undergo a process of continual transformation. They exhibit a state of unstable equilibrium in respect to the opposing tendencies toward a deposit of new cartilage on the one hand and toward the excavation of the old on the other. The margins thereby are always either advancing or receding, and it is in this way that the progressive alterations in the size, shape, and position of the cavities are produced, due to which a suitable suite of chambers is always provided for the enlarging membranous labyrinth.

The general tissue mass of the otic capsule, during the period represented by embryos from 4 to 30 mm. long, passes through three consecutive histogenetic periods, namely, the stage of mesenchymal syncytium, the stage of precartilage, and the stage of true cartilage. In the subsequent growth of the capsule it is found that in areas where new cartilage is being deposited the tissues of the areas concerned follow a definite and progressive order of development. In areas, however, where excavation occurs, where cartilage previously laid down is being removed, it is found that the process is reversed. The tissue in such areas returns to an earlier embryonic state—that is, it undergoes dedifferentiation. Tissue that has acquired all the histological characteristics of true cartilage can thus be traced in its reversion to precartilage and from precartilage in turn to a mesenchymal syncytium. In the latter form it redifferentiates into a more specialized tissue, in this case for the most part into a vascular reticulum.

The formation of the periotic reticulum is first indicated by a cluster of deeply staining nuclei that can be seen along the central edge of the semicircular ducts in embryos soon after the ducts are formed, and at about the time the otic capsule begins to change from condensed mesenchyme into precartilage. These nuclei constitute a focus at which the development of the reticulum and its blood-vessels takes origin. Here the tissue of the otic capsule takes on an appearance that is less like that of a cartilage-forming tissue and more like that of an embryonic connective tissue. Spreading from this focus, a narrow area is established which soon encircles the semicircular ducts and becomes the open-meshed vascular reticulum which, in embryos 30 mm. long, everywhere bridges the space existing between the epithelial labyrinth and the surrounding cartilage. In the earlier stages it could not be definitely shown that the primordium of the periotic reticular tissue is not derived from a few predestined mesenchyme cells which become inclosed, along with the otic vesicle, by the condensed tissue of the capsule and after a certain latent

period undergo proliferation and occupy the space vacated by the receding precartilage. In the later stages, however, it is quite evident that precartilage tissue is actually converted into a reticulum, and that the replacement of precartilage by a reticular connective tissue is brought about through a process of dedifferentiation.

The perichondrium is a derivative of the periotic reticulum and forms an outer limiting membrane along its cartilaginous margin. During the fetal period the perichondrium does not rest directly against the true cartilage, but is separated from it by a zone of transitional tissue consisting partly of precartilage and partly of reticulum. This transitional zone intervening between the perichondrium and the surrounding cartilage was observed in all of the specimens that were studied, which includes fetuses up to 130 mm. crown-rump length. Owing to the fact that the perichondrium is late in making its appearance, being first seen in fetuses about 70 mm. long, it can take no part in the early changes in the cartilaginous capsule, either as regards the deposit of new cartilage or the excavation of cartilage that had been previously laid down.

The periotic tissue-spaces are formed by a modification of the meshes of the periotic reticulum. The latter consists originally of a rather uniform narrow mesh. The essential change which it undergoes in the process of space-formation consists in the gradual disappearance of the traversing trabeculæ. The trabeculæ consist of the protoplasmic processes of the constituent cells of the reticulum, and their disappearance is apparently due, not to a dissolution or liquefaction of these cell-processes, but to an alteration in their form. It apparently is the result of an active motility of the cell protoplasm involving the successive detachment and retraction of the trabeculæ. When a trabecula becomes detached it retracts and adapts itself to the formation of the enlarging space, reshaping itself either as a smooth border or as a constituent part of another trabecula.

The differentiation of the margin of the periotic spaces constitutes the final feature in their maturation. During the period in which the enlargement of an individual space is actively going on, the margins of the main cavity consist of smooth, delicate strands of nucleated protoplasm that resemble the trabeculæ between the large reticular spaces. These linear margins are interrupted here and there by openings into adjacent spaces. They tend, however, to form a continuous line that definitely marks off the space from the adjacent reticulum. As the space becomes more mature, the membrane-like border becomes thicker until it reaches a state that will probably not admit of any further opening-up for the coalescence of additional spaces. Any further growth is thereafter limited to a simple distention of the wall of the space, with the consequent adjustment of its constituent cells. In its final form the margin of the space constitutes a mesothelial membrane. Immediately lining the space is a thin membrane with flattened nuclei which is supported underneath by a thin coat of nucleated protoplasm having the form of fibrous connective tissue. The former in its histogenesis differs in no way from the rest of the wall and the difference that exists later seems to be merely the result of its adaptation to the existing physical conditions.

The earliest histological evidence of the formation of the periotic spaces occurs near the stapes, in the reticulum that bridges the interval between the sacculus and the fenestra vestibuli. In embryos between 30 mm. and 40 mm. long, it can be seen that the meshes in this region are becoming irregular and larger, due to the disappearance of some of the trabeculæ and a consequent coalescence of the intertrabecular spaces. The widening of the mesh at this point constitutes the primordium of the vestibular cistern. It makes its appearance before there is any trace of the scalæ, but it is not until the fetus reaches a length of about 50 mm. that the cistern becomes definitely outlined and clearly differentiated from the adjoining reticulum.

Following the appearance of the cistern, the scala tympani is the next space to become established. It can be recognized as a moderate widening of the meshes of the reticulum in the region of the fenestra cochleæ in fetuses 43 mm. long, along the basal border of the first turn of the cochlear duct. The scala vestibuli, as can be seen in fetuses 50 mm. long, develops as an extension downward of the cistern along the apical border of the cochlear duct. Starting from these definite foci, these three spaces spread into their destined territory, absorbing as they go the enlarging reticular spaces of the invaded region by a process of space-coalescence, or, in other words, the progressive formation of areas that are free of trabeculæ. In fetuses 85 mm. long the two scalæ extend downward along the cochlear duct to its last turn, as two separate spaces which do not communicate with each other. When they reach the tip of the duct, which occurs in fetuses about 130 mm. crown-rump length, a free opening is developed between them which represents the helicotrema. After being completely established along the whole length of the cochlear duct, the scalæ continue to enlarge by further coalescence of tissue along their peripheral border, in which the trabeculæ disappear.

The periotic spaces are analogous in their development to the pia-arachnoidal spaces; they are not, however, extensions of them that have invaded the cavity of the cartilaginous labyrinth. They begin at points where there can be no connection with the arachnoidal tissue and their direction of growth is quite independent of it. The communication that is found in the adult between the scala tympani and the subarachnoid space in the neighborhood of the fenestra cochleæ, the so-called aquæductus cochleæ, is established quite late. In fetuses 85 mm. crown-rump length it exists as a tubular pouch projecting from the subarachnoid spaces along the glossopharyngeal nerve toward the scala tympani. In the 130-mm. fetus, the oldest examined, this pouch is longer and nearly reaches the scala. The communication must be established soon after this.

Similar projections from the subarachnoid spaces at the internal auditory meatus extend as perineural clefts along the trunk and branches of the acoustic nerve. No actual communications, however, were seen between these spaces and the two scalæ.

BIBLIOGRAPHY.

Balfour, F. M., 1885. A treatise on comparative embryology. 2 ed., vol. 2. London.

Bardeen, C. R., 1910. Die Entwicklung des Skeletts und des Bindegewebes. Keibel-Mall, Handbuch der Entwicklungsgeschichte, I, 296–456.

Boettcher, A., 1869. Ueber Entwickelung und Bau des Gehörlabyrinths nach Untersuchungen an Säugethieren. Dresden. (Quoted from Retzius, 1884.)

———, 1872. Kritische Bemerkungen und neue Beiträge zur Literatur des Gehörlabyrinths. Dorpat. (Quoted from Retzius, 1884.)

Breschet, G., 1836. Recherches anatomiques et physiologiques sur l'organe de l'ouïe et sur l'audition dans l'homme et les animaux vértébrés. 2 ed. Paris.

Cassebohm, J. F., 1735. Tractatus anatomici de aure humana, etc. Halae, Magdeburgicæ. (Quoted from Breschet, 1836.)

Child, C. M., 1915. Senescence and rejuvenescence. University of Chicago Press.

Corti, A., 1851. Recherches sur l'organe de l'ouïe des mammifères. Première partie. Zeitschr. f. wiss. Zool., III, 109–169.

Cotunnii, D., 1760. De aquæductibus auris humanæ internæ anatomica dissertatio. Napoli. (Quoted from Breschet, 1836.)

von Ebner, V., 1902. Kölliker's Handbuch der Gewebelehre. Bd. III. 2, 6 Aufl.

Filatoff, D., 1906. Zur Frage über die Anlage des Knorpelschädels bei einigen Wirbeltieren. Anat. Anz., XXIX, 623–633.

Gaupp, E., 1906. Die Entwickelung des Kopfskelettes. Hertwig's Handbuch der Entwick. d. Wirbeltiere.

Hasse, C., 1873. Die Lymphbahnen des inneren Ohres der Wirbelthiere. *In his* Anatomische Studiem. Leipzig., I, Heft 4, No. 19, 766–816.

———, 1881. Bemerkungen über die Lymphbahnen des inneren ohres. Arch. f. Ohrenh., XVII, 188–194.

Hensen, V., 1863. Zur Morphologie der Schnecke des Menschen und der Säugethiere. Ztschr. f. wiss. Zool., XIII, 481–512.

His, W., 1903. Die Häute und Höhlen des Körpers. Wiederabdruck eines akademischen Programmes vom Jahre 1865. Arch. f. Anat. u. Physiol., Anat. Abth., 368–404.

Huschke, E., 1831. Erste Bildungsgeschichte des Auges und Ohres. (Versammlung Naturforscher u. Aerzte zu Hamburg.) Isis von Oken, 950.

Key, E. A. H., and G. Retzius, 1875–1876. Studien in der Anatomie des Nervensystems und des Bindegewebes. Stockholm.

Kölliker, A., 1850–1854. Mikroskopische Anatomie oder Gewebelehre des Menschen. Leipzig.

———, 1861. Der embryonale Schneckenkanal und seine Beziehungen zu den Theilen der fertigen Cochlea. Würzburger naturwiss. Zeitschr., II, 1–9.

———, 1861. Entwicklungsgeschichte des Menschen und der höheren Thiere. Leipzig.

———, 1879. Entwicklungsgeschichte des Menschen und der höheren Thiere. 2 ed., Leipzig.

———, 1884. Grundriss der Entwicklungsgeschichte des Menschen und der höheren Thiere. 2 ed., Leipzig.

Krause, R., 1906. Entwickelungsgeschichte des Gehörorganes. Handbuch d. vergl. u. exper. Entwicklungslehre der Wirbeltiere. Herausg. v. O. Hertwig. Jena.

Levi, G., 1900. Beitrag zum Studium der Entwickelung des Knorpeligen Primordial craniums des Menschen. Archiv f. mikrosk. Anat., Bd. 55

Lewis, W. H., 1907. On the origin and differentiation of the otic vesicle in amphibian embryos. Anat. Rec., I, 141.

———, 1915. The use of guide planes and plaster of paris for reconstruction from serial sections: Some points on reconstruction. Anat. Rec., IX, 719.

Macklin, C. C., 1914. The skull of a human fetus of 40 mm. Amer. Jour. Anat., vol. 16.

Mall, F. P., 1902. On the development of the connective tissues from the connective tissue syncytium. Amer. Jour. Anat., I, 329–365.

Morgagni, J. B., 1740. Epist. anatom., XII, p. 469. Venetiis. (Quoted from Breschet, 1836.)

Odenius, M. V., 1867. Ueber das Epithel der Maculae acusticae beim Menschen. Arch. f. mikr. Anat., III, 115–134.

Reichert, C. B., 1852. Bericht über die Abhandl. d. Herrn Dr. Reissner "De auris internæ formatione." Bull. d. la Cl. phys.-math. d l'acad. imp. d. sci. d. St. Pétersbourg, X, 86–96.

———, 1864. Beitrag zur feineren Anatomie der Gehörschnecke des Menschen und der Säugethiere. Abhandl. d. Kgl. Akad. d. Wiss., Berlin, (Phys.) No. 1, 1–60.

Reissner, E., 1851. De auris internæ formatione. Inaug. diss., Dorpat. (See Reichert, 1852.)

———, 1854. Zur Kenntniss der Schnecke im Gehörorgan der Säugethiere und des Menschen. J. Müller's Arch. f. Anat., Physiol. u. wiss. Med. 420–427.

Retterer, E., 1900. Évolution du cartilage transitoire. Jour. de l'Anat. et de la Physiol., XXXVI, 467–565.

Retzius, G., 1884. Das Gehörorgan der Wirbelthiere. Abtheilung II. Stockholm.

Sabin, F. R., 1917. Origin and development of the primitive vessels of the chick and of the pig. Contributions to Embryology, vol. 6, No. 15. Carnegie Inst. Wash. Pub. No. 226.

Scarpa, A., 1789. Anatomicæ disquisitiones de auditu et olfactu. Ticini. (Quoted from Retzius, 1884.)

Schwalbe, G., 1869. Der Arachnoidalraum ein Lymphraum und sein Zusammenhang mit dem Perichorioidalraum. Centralbl. f. d. med. Wiss., No. 30, 465–467.

Solger, B., 1889. Ueber Knorpelwachstum. Verh. d. Anat. Gesellsch. Berlin, III, 67–71. (Ergänzungsheft Anat. Anz., IV.)

Streeter, G. L., 1917. The factors involved in the excavation of the cavities in the cartiliginous capsule of the ear in the human embryo. Amer. Jour. Anat., XXII, 1–25.

———, 1917. The development of the scala tympani, scala vestibuli, and perioticular cistern in the human embryo. Amer. Jour. Anat., XXI, 299–320.

Terry, R. J., 1917. The primordial cranium of the cat. Jour. Morph., vol. 29.

Valsalva, A. M., 1707. De aure humana tractatus, etc. Imolensi, Trajecti ad Rhenum. (Quoted from Breschet, 1836.)

Vieussens, R., 1714. Traité nouveau de la structure de l'oreille. Toulouse. (Quoted from Breschet, 18 6.)

Weber-Liel, 1879. Der Aquæductus cochleæ des Menschen. Monatschr. f. Ohrenh., XIII, 33–39.

Weber, F. E., 1869. Ueber den Zusammenhang des Arachnoidalraumes mit dem Labyrinth. Monatschr. f. Ohrenh., III, 105–107.

Weed, L. H., 1917. The development of the cerebrospinal spaces in pig and in man. Contributions to Embryology, vol. 5, No. 14. Carnegie Inst. Wash Pub. No. 225.

EXPLANATION OF PLATES.

Plate 1.

The figures on Plates I and II represent a series of photographs of the ear region in human embryos varying from 4 mm. to 130 mm. long. The photographs were taken at a magnification of 100 diameters and as far as possible at similar positions, so that a comparison of them would indicate the actual increase in size and the relative amount and form of the individual tissue-masses. In the reproduction they were reduced to about 90 diameters. The different figures include the principal stages in the development of the cartilaginous capsule of the ear and show the gross features of the histogenesis of the periotic reticulum. Figures 5 to 7 cover the period during which the mesenchyme becomes condensed around the otic vesicle. Figures 8 to 10 show the otic capsule in its precartilage stage and the manner in which the precartilage becomes differentiated into relatively permanent and temporary zones. The latter encircle the epithelial ducts and correspond to the future cartilaginous canals. In figures 11 to 13 the main capsular mass has become true cartilage, whereas the temporary zone of precartilage surrounding the canal is on the point of dedifferentiating into periotic reticulum. A focal area of vascularized reticulum is already established at the inner margin of the epithelial duct.

Fig. 5. Frontal section through the region of the ear in a human embryo 4 mm. long (Carnegie Collection, No. 588, slide 6, row 6, section 6). The section is 15μ thick and is enlarged 90 diameters. It shows part of the brain-wall and the otic vesicle with the surrounding mesenchyme. The nuclei of the latter are more numerous in the neighborhood of the vesicle, indicating the beginning of the capsular condensation.

Fig. 6. Horizontal section through the region of the ear in a human embryo 9 mm. long (Carnegie Collection, No. 721, slide 5, row 2, section 1). The section is 15μ thick and is enlarged 90 diameters. It shows a distinct condensation of the mesenchyme around the otic vesicle, particularly on its lateral surface (above) where it extends from the surface of the vesicle to about half the distance from the vesicle to the ectoderm.

Fig. 7. Frontal section through the labyrinth in a human embryo 11 mm. long (Carnegie Collection, No. 353, slide 16, row 3, section 4). The section is 10μ thick and is enlarged 90 diameters. It shows the vestibular part of the labyrinth with the appendage opening out of it and passes transversely through the pouches whose margins are to form the superior and lateral semicircular ducts. There is now a very complete capsule of condensed mesenchyme surrounding every part of the labyrinth, with the exception of the appendage and the regions of the internal auditory meatus and the fenestra cochleæ.

Fig. 8. Horizontal section through the otic capsule in a human embryo 15 mm. long (Carnegie Collection, No. 719, slide 3, row 2, section 3). The section is 40μ thick and is enlarged 90 diameters. It shows a portion of the utricle below and the superior semicircular duct above. Surrounding these is a definite capsule of precartilage tissue.

Fig. 9. Sagittal section through the otic capsule in a human embryo 18 mm. long (Carnegie Collection, No. 144, slide 4, row 1, section 3). The section is 40μ thick and is enlarged 90 diameters. Above is the posterior semicircular duct, and just below the center is the lateral semicircular duct. The otic capsule is now differentiated into relatively permanent areas of precartilage and other areas that are more temporary. The latter surround the epithelial ducts and indicate the future cartilaginous canals.

Fig. 10. Frontal section through the otic capsule in a human embryo 27 mm. crown-rump length (Carnegie Collection, No. 756 *a*, slide 47, section 2). The section is 50μ thick and is enlarged 90 diameters. It passes transversely through the lateral semicircular canal. The epithelial duct is surrounded by a zone of temporary precartilage corresponding to the future cartilaginous canal. Just median to the duct (below it in the photograph) is a group of nuclei that forms the focus of the future growth of reticulum.

Fig. 11. Section through the lateral semicircular canal in a human fetus 30 mm. crown-rump (Carnegie Collection, No. 86, slide 46, section 2). The section is 50μ thick and is enlarged 90 diameters. The main capsular mass is now differentiated into true cartilage. The zone of temporary precartilage is beginning to recede from the epithelial duct, leaving a reticular area in the interval, which is more pronounced on the median side of the duct (below it in the photograph).

Fig. 12. Section through the lateral semicircular canal in a human fetus 37 mm. crown-rump length (Carnegie Collection, No. 972, slide 20, section 1). The section is 50μ thick and is enlarged 90 diameters. The nuclei of the zone of temporary precartilage form a dark field that corresponds to the future cartilaginous canal. Along the inner margin of this zone are seen large blood-vessels that belong to the periotic reticulum.

Fig. 13. Section through the lateral semicircular canal in a human fetus 35 mm. crown-rump length (Carnegie Collection, No. 199, slide 58, section 2). The section is 50μ thick and is enlarged 90 diameters. It is stained deeply with hematoxylin, showing the matrix of the cartilage but not the zone of precartilage that is to become the cartilaginous canal.

Plate 2.

The figures on Plate II are in continuation of those on Plate I and show the final establishment of the periotic reticular tissue. They also show, on being compared with younger stages, the manner in which the cartilage becomes excavated in order to yield room for the enlarging duct and also to allow for its changing position. The excavation is brought about by the dedifferentiation of cartilage into reticular tissue. Throughout this period the margin of the cartilaginous canal continues in an unstable condition and is gradually either

receding or advancing, through the processes of dedifferentiation, into precartilage or differentiation from precartilage respectively. The periotic reticulum in its later stages develops fibrous membranes at its inner and outer borders. The one at the inner border forms the membrana propria for the epithelial duct, and the one at the outer border becomes the perichondrium.

FIG. 14. Section through the lateral semicircular canal in a human fetus 43 mm. crown-rump length (Carnegie Collection, No. 886, slide 42, section 3). The section is 100μ thick and is enlarged 90 diameters. The zone of precartilage is expanding around its peripheral margin by dedifferentiation of the surrounding cartilage and on its central margin the precartilage is giving way before the advancing reticulum. A crescentic area of periotic reticulum is established on the median side (to the left) of the epithelial duct, about 8 mm. deep in the photograph.

FIG. 15. Section through the lateral semicircular canal in a human fetus 46 mm. crown-rump length (Carnegie Collection, No. 95, slide 72, section 1). The section is 100μ thick and is enlarged 90 diameters. The original area of precartilage is now all dedifferentiated into reticulum, and a new area of precartilage has formed outside of this at the expense of the surrounding cartilage. The new area of precartilage is about 0.8 cm. deep in the photograph. Everything between this and the epithelium is reticulum, the peripheral part of which is not yet completely vascularized.

FIG. 16. Section through the posterior semicircular canal in a human fetus 50 mm. crown-rump length (Carnegie Collection, No. 184, slide 23). The section is 50μ thick and is enlarged 90 diameters. The dedifferentiation of precartilage into reticulum is nearly complete, there being left only a narrow line of it along the margin of the cartilage. The vascularization of the reticulum is not yet completed. The small diameter and the thick wall of the epithelial duct in this figure and in figure 15 result from contraction. If they were distended in the process of fixation they would doubtless be as large as those in figures 14 and 17.

FIG. 17. Section through the posterior semicircular canal in a human fetus 52 mm. crown-rump length (Carnegie Collection, No. 96, slide 12, section 2). The section is 100μ thick and is enlarged 90 diameters. It differs from figure 16 in having a more mature periotic reticulum.

FIG. 18. Section through the posterior semicircular canal in a human fetus, 85 mm. crown-rump length (Carnegie Collection, No. 1400–30, slide 43, section 2). The section is 100μ thick and is enlarged 90 diameters. At the inner margin of the reticulum can now be seen the membrana propria supporting the semicircular duct and at the outer margin is the thick perichondrium, between which and the cartilage there is a narrow open space that is better seen on the left part of the photograph. The sharp dark line along the margin of the cartilage on the right is an appearance due to the excavation of cartilage at that point. It consists of an intermediate zone in which the cartilage is being dedifferentiated into precartilage and that in turn into reticular tissue.

FIG. 19. Section through the superior semicircular canal in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 30, section 1). The section is 50μ thick and is enlarged 90 diameters. It shows a rather mature perichondrium closely attached to the cartilage, separated from it, however, by a narrow intermediate zone that is not seen in the photograph. This zone is connected with the further enlargement of the cartilaginous canal, the growth of which is not yet completed. In the outer part of the canal the perichondrium fuses with the membrana propria of the semicircular duct. The periotic reticulum is beginning to break up in the formation of larger spaces, which it does by the retraction of its trabeculæ, thereby allowing adjacent spaces to coalesce. The blood-vessels in this specimen were injected with India ink.

PLATE 3.

The figures on Plate III show the histological appearance of the periotic tissue-spaces and the manner in which they are formed from the periotic reticulum. This is accomplished by the disappearance of the trabeculæ and the consequent repeated coalescence of adjoining spaces.

FIG. 20. Section through the second turn of the cochlea in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 32, section 2), enlarged 57 diameters. This section shows the topography of the cochlear duct and the general character of the periotic spaces that are developing along its inner margins. Details of this same section as seen under higher magnification are shown in figures 22 and 24.

FIG. 22. Detail of the section shown in figure 20, enlarged 278 diameters. This figure shows the part of the cochlear duct that is to form the organ of Corti with the adjacent tissue that becomes incorporated in the basilar membrane. Below is the periotic reticulum, whose spaces are in the process of enlarging. By repeated coalescence these spaces finally unite with the large space which constitutes the scala tympani. This figure shows the histological appearance of the reticulum where the formation of tissue-spaces is in active operation.

FIG. 24. Detail of the section shown in figure 20, enlarged 300 diameters. It shows the character of the margin of the scala vestibuli in a fairly mature condition. The scala vestibuli is inclosed by a membrane consisting of the cells that had previously constituted the reticulum occupying this area and which have been modified in form in adaptation to the formation of this large tissue-space, closing it off from the surrounding tissue.

FIG. 21. Section through the vestibular portion of the labyrinth in a human fetus 52 mm. crown-rump length (Carnegie Collection, No. 448, slide 154, section 2), enlarged 31 diameters. This section shows the general character of the periotic spaces and their relation to the different parts of the membranous labyrinth and the surrounding cartilaginous capsule. The first space to develop and the largest shown in this figure is the vestibular cistern, situated between the utricle and the cartilaginous stapes. The smaller spaces, below the cistern and extending downward along the cochlear duct, represent the scala vestibuli in an early form. The arteries in this specimen were injected with India ink and are shown in black.

FIG. 23. Section through the superior semicircular canal in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 29, section 2), enlarged 90 diameters. The periotic reticulum is undergoing the alterations characteristic of the early stages of the formation of tissue-spaces. Along the margins of the cartilage the reticular tissue is condensed and constitutes the fibrous perichondrium. Around the epithelial canal there is developed a layer of supporting tissue which forms the membrana propria. This layer fuses with the perichondrium along the peripheral margin of the canal and thereby constitutes a ligament that attaches each membranous duct throughout its whole length to the cartilaginous space in which it is suspended.

FIG. 25. Section through the apex of the cochlea of a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018, slide 32, section 2), enlarged 57 diameters. This section shows the tip of the cochlear duct and the character of the communication that develops between the two scalæ forming the helicotrema. It will be seen that the margins of the periotic spaces are not so mature here as in the proximal parts of the cochlea of the same fetus, on comparing this figure with figure 20.

PLATE 4.

The figures shown on this plate represent a series of median and lateral views of wax-plate reconstructions of the membranous labyrinth and the surrounding periotic tissue-spaces. They illustrate under the same scale of enlargement three typical stages in the development of these spaces. Abbreviations: C. s. l., ductus semicircularis lateralis; C. s. p., ductus semicircularis posterior; C. s. s., ductus semicircularis superior; Duct. coch., ductus cochlearis; Impressio rotund., area opposite the fenestra cochleæ; Impressio staped., area in contact with base of stapes; Saccus endol., saccus endolymphaticus; Scala tymp., scala tympani; Scala vestib., scala vestibuli.

FIG. 26. Lateral view of a model reconstructed from a human fetus 50 mm. crown-rump length (Carnegie Collection, No. 84). The cistern and the scala vestibuli are shown in green and the scala tympani is shown in orange. The scala vestibuli is in the first stage of its development and consists of a row of large reticular spaces which extend from the ventral margin of the cistern downward along the apical surface of the cochlear duct. The scala tympani is more advanced and shows more complete coalescence of its constituent spaces. Enlarged 11.4 diameters.

FIG. 27. Median view of the same model shown in figure 26. This view shows the topography of the scala tympani. Its large proximal end lies opposite the fenestra cochleæ (rotunda) and corresponds to the focus at which its development originates. Distally it tapers off rapidly where the spaces are smaller and their coalescence less complete. Enlarged 11.4 diameters.

FIG. 28. Lateral view of wax-plate reconstruction of the left membranous labyrinth and the periotic spaces in a human fetus 85 mm. crown-rump length (Carnegie Collection, No. 1400–30), enlarged 11.4 diameters. The cistern and the connecting scala vestibuli are shown in green. Although the greater part of the cistern abuts against the stapes, it will be noted that it is also beginning to spread over the dorsal surface of the utricle and along the inner border of the lateral semicircular duct. The scala vestibuli communicates freely with the cistern and extends downward along the apical surface of the cochlear duct throughout nearly two turns, showing the characteristic sacculated appearance near its tip, where the coalescence of the spaces is less complete.

FIG. 29. Median view of same model shown in figure 28, enlarged 11.4 diameters. The scala tympani is shown in orange. The oval indentation in its proximal end corresponds to the fenestra cochleæ (rotunda). This space extends along the cochlear duct about the same distance as the scala vestibuli, but the two do not communicate yet at any place. The peripheral border of the scala tympani is characterized by sacculations corresponding to spaces that are coalescing with the main space. The growth of the scala is due to a coalescence of new spaces along its peripheral border rather than along its central border.

FIG. 30. Lateral view of a wax-plate reconstruction of the left membranous labyrinth and the periotic spaces in a human fetus 130 mm. crown-rump length (Carnegie Collection, No. 1018), enlarged 11.4 diameters. The cistern and scala vestibuli are shown in green and the scala tympani is shown in orange, as in the previous figures. The cartilaginous stapes was removed from this model and the oval impression that it makes on the cistern can be plainly seen. The cistern has spread over the top of the utricle and part way along the lateral semicircular duct. The scala vestibuli extends to the tip of the cochlear duct, where it communicates with the scala tympani, thus forming the helicotrema.

FIG. 31. Median view of same model shown in figure 30, enlarged 11.4 diameters. The oval impression on the proximal end of the scala tympani corresponds to the fenestra cochleæ (rotunda). As yet there is no communication at this point between the scala tympani and subarachnoid spaces, such as is found in the adult and known as the aquæductus cochleæ. The spaces making up the cistern cover almost the whole of the utricle and saccule except the places at which the nerves enter and a small part of the medial surface near the attachment of the appendage.


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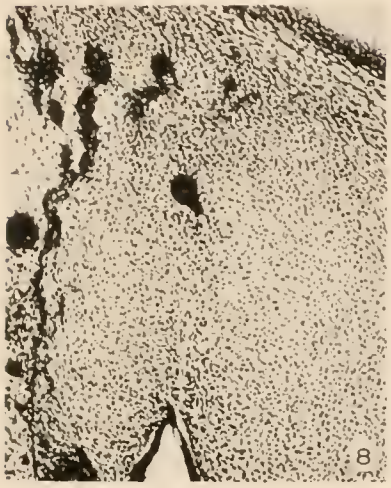
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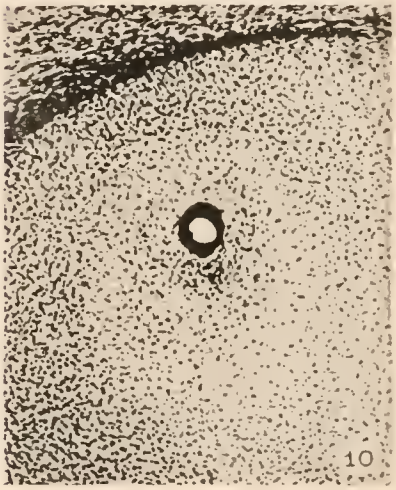
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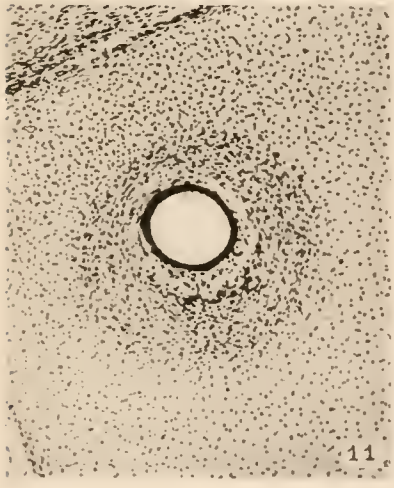
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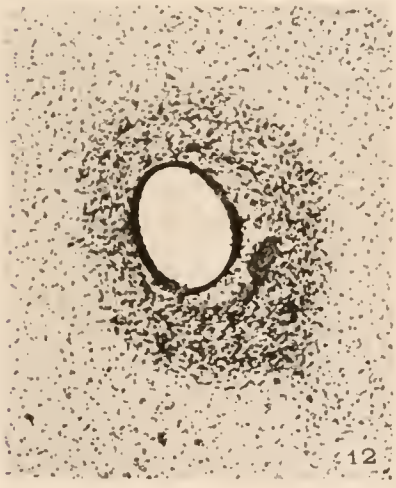
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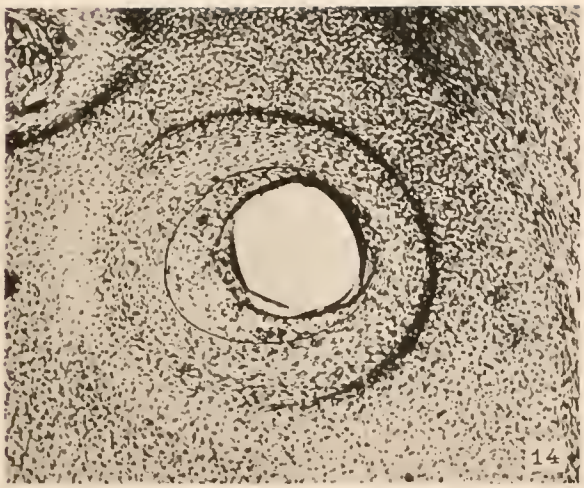
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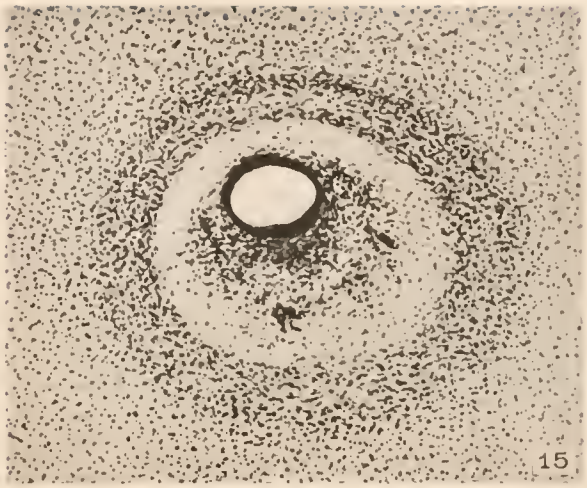
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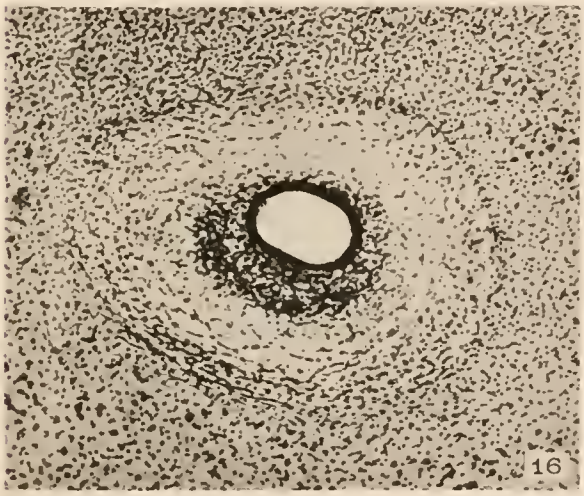
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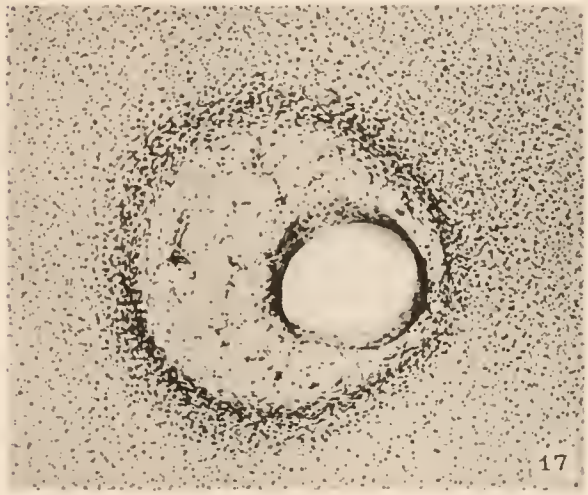
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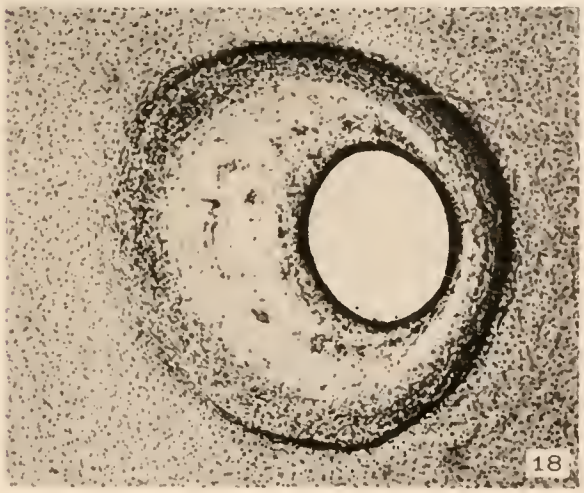
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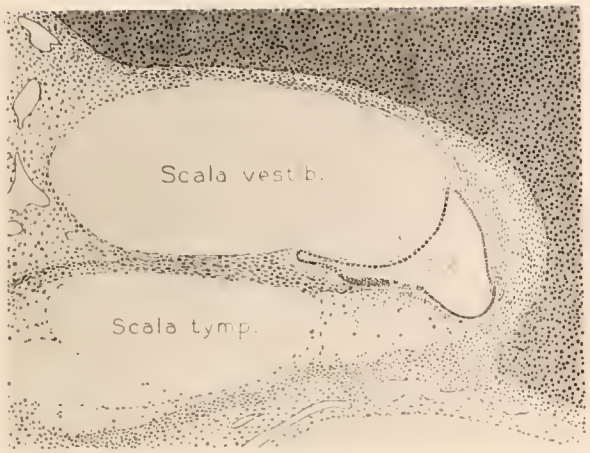

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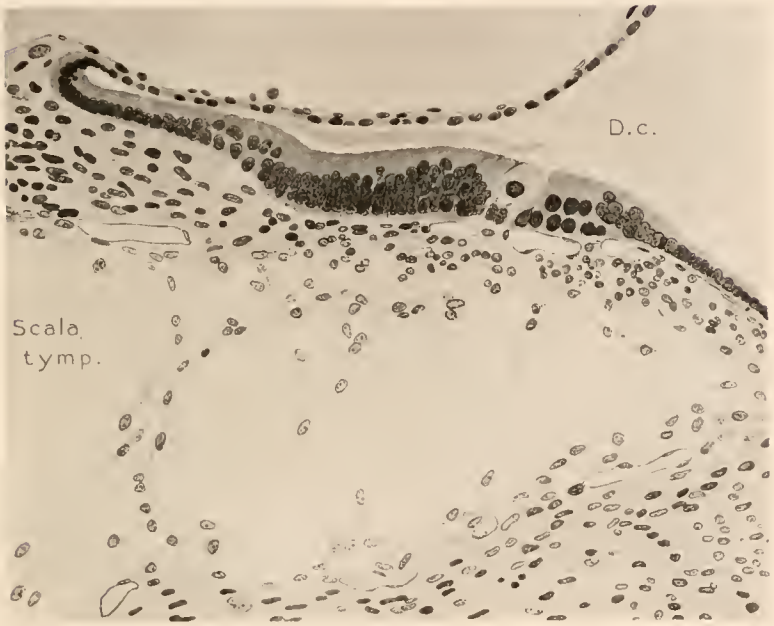

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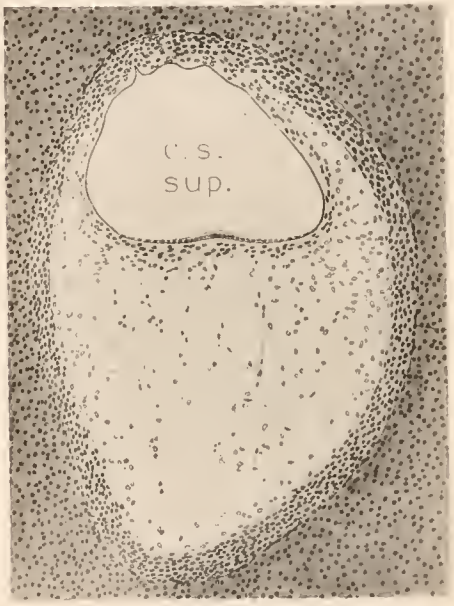

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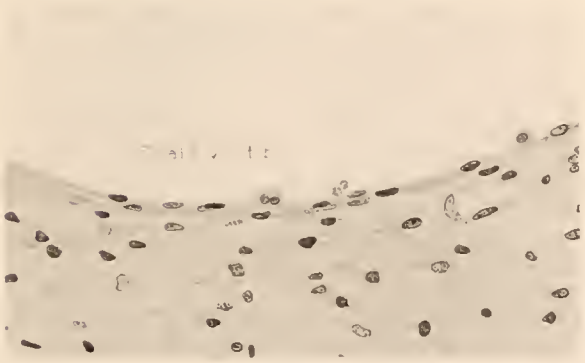

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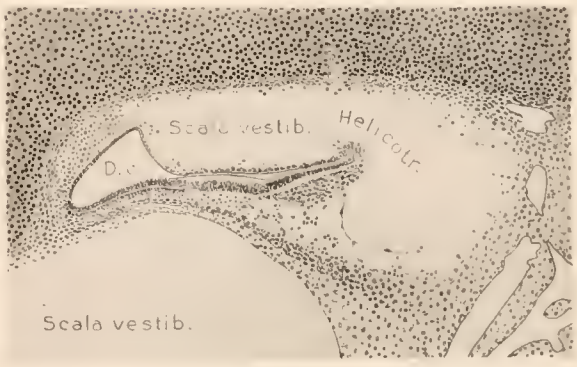

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J. F. Didusch fec.

Albert & Co Lith.

CONTRIBUTIONS TO EMBRYOLOGY, No. 21.

THE GENESIS AND STRUCTURE OF THE MEMBRANA TECTORIA AND THE CRISTA SPIRALIS OF THE COCHLEA.

By O. Van der Stricht.

With four plates (or thirty-six figures).

THE GENESIS AND STRUCTURE OF THE MEMBRANA TECTORIA AND THE CRISTA SPIRALIS OF THE COCHLEA.

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INTRODUCTION.

The membrana tectoria belongs to a group of organs produced at the surface of the epithelium and termed superficial cuticles or superficial cuticular formations. Once developed, the generating epithelium persists in its entirety beneath the cuticle or exceptionally may disappear, as in the case of the ameloblasts, which atrophy after forming the enamel at their bases.

One may subdivide these structures into three groups: In the first the process of development can not be doubted or denied. It occurs just within the superficial layer of the cytoplasm and the cuticle produced remains in close contact, even continuity, with the generating cells. Examples are the striated borders of the columnar epithelium of the intestine, of the crypts of Lieberkühn, of the convoluted tubules of the kidney, of the syncytial layer of chorionic villi in the human placenta, and of osteoclasts.

The second is represented by the series of reticulares or fenestrated membranes covering the surface of sensorial epithelia—for example, the reticular membrane of the crista—and macula acustica and the organ of Corti, the membrana limitans externa of the retina, the membrana limitans olfactoria. The openings of the membrane are traversed by the apices of sensorial cells, the hairs of the acoustic cells, the rods and cones of the retina, the ciliated vesicles of the olfactory cells. These membranes in adult sensorial organs are in close contact with the surface of the epithelium, but are completely separated from the generating substratum. Hence their origin must be studied during the embryonic period of their development. Many authors regard them as a real cuticle derived from the free surfaces of the subjacent, that is to say, sustentacular cells. N. Van der Stricht (1908) has demonstrated that the reticular membrane of the acoustic epithelium is formed by a system of terminal bars closing the intercellular spaces between the embryonic epithelial cells. G. Leboucq (1909) proved that the membrana limitans externa of the retina is not formed at all by the Müller cells, but by the closing bars separating the apices of the rods and cones and Müller cells. The present writer (1909) found the membrana limitans olfactoria to have a similar origin. The zona pellucida surrounding the ovarian ovum in mammals and traversed by the prolongations of follicular cells which reach the surface of the egg must be considered as a fenestrated membrane of the same nature. According to the investigations of Dubreuil and Regaud (1908), it is derived from exoplasmic fibers produced within the intercellular spaces of the follicular cells. My own preparations of ovaries of

bats and dogs show that it is formed by the terminal bars, and Alice Thing (1917) considers that the very thick zona pellucida of the ovum of the turtle is produced by the terminal bars of the surrounding epithelium. They extend over the free surfaces of these cells, where appears a delicate network of the same nature as that of the bars. This network, together with the bars, gives rise to the cuticular fundamental substance of the zona pellucida.

Enamel and the membrana Corti or membrana tectoria are included in a third group. In both cases the adult organ becomes completely detached from its generating substratum, the first from the bases of the ameloblasts, each of which produces a kind of cuticular prism (the enamel prism). These elements are separated by the calcified cement substance which is considered to be a kind of intercellular product, although its origin has not been clearly described. The second, the membrana tectoria, becomes detached from the surface of the greater and the lesser epithelial ridges in the cochlear duct and remains fixed to only the least active portion of its generating substratum, the crista spiralis. Held (1909), discussing the nature of the membrana Corti, thinks that the membrane should not be considered cuticular, not because its layer first formed is not homogeneous, but because its constituent elements, its fibrils, as they become more and more elongated, proceed from the cytoplasm as different plasmic products and not as cell prolongations. A cuticle, he states, is not represented by flagella, by cilia of a ciliated epithelium, or by sensorial hairs. In addition a cuticle always remains attached to the surface of the cell. Hence Held regards the membrana tectoria as a specific product of the free surface of the greater and lesser epithelial ridges, the sensorial cells of which do not take part in its development. Therefore the fibrils of the membrana Corti can not be termed cuticular. Held seems to forget the recognized fact that enamel prisms are real cuticular elements, although they become completely detached from their anatomical substratum.

The object of my research is the study of the development and structure of the membrana tectoria. Although this problem has received the attention of many investigators, it seems to me that the results obtained have been rather contradictory and give for the most part no satisfactory interpretation because of differences between the morphological substratum and the real structure of the membrane derived from it. Recent investigators have more or less neglected the structure of the crista spiralis. I intend to devote to it special attention.

METHODS.

I have investigated the following material: Pig embryos of 60.0, 93.5, 95.0, 127.0, 137.0, 150.0, and 190.0 mm.; a new-born dog; young kittens; the following adult animals: bat, dog, rat, and mouse.

The isolated cochlea was fixed by one of the following agents: Trichloracetic acid, 5 per cent in water; this decalcifies bone very well after one, two, or three days, according to the size of the cochlea; Bouin's fluid; Zenker's fluid.

After fixation by the first agent, the pieces were transmitted directly to absolute alcohol, to which some drops of iodine had been added. After remaining one

or two days in the second or third fluid, the pieces were washed in running water and hardened for many weeks in 70 per cent alcohol with some drops of iodine. The iodine effectively acts as a mordant. Where necessary, decalcification was completed by 2 per cent nitric acid in 70 per cent alcohol.

Before embedding in paraffin the pieces were stained by borax carmine. The series was then stained by iron hematoxylin, Congo red, and light green. In advanced stages of development the best results were usually obtained by the following treatment:

1. Immerse one day in 2.5 gm. ferric alum in 100 c.c. distilled water.
2. Wash one second in distilled water.
3. Immerse for some minutes in a solution of Congo red, 1 gm. in distilled water 200 c.c.
4. Wash in distilled water.
5. Immerse one day in a 0.5 per cent aqueous solution of crystallized hematoxylin.
6. Decolorize by a 1 per cent aqueous solution of ferric alum.
7. Wash for one hour in running water.
8. Stain for some seconds in a solution of 0.5 gm. light green in 200 c.c. of 95 per cent alcohol.
9. Treat in succession with absolute alcohol, xylol, and Canada balsam.

By this method the nuclei, the central corpuscles, and the terminal bars are stained very dark blue, the cytoplasm and its prolongations red, the ground substance of the connective tissue and the membrana tectoria green.

Mallory's method is also very useful for staining blue the ground substance of the connective tissue and the membrane of Corti.

The membrana tectoria possesses very delicate structures in which shrinkage and agglutination are provoked by the best fixing agents, although some of my series give results which are good and are confirmed by a new and better method tried during the past few weeks. Before treatment by one of the three fixing fluids mentioned above, I made one or two small openings in the bony wall of the cochlea and exposed the piece for 15 minutes to vapors from an aqueous solution of osmic acid or submerged it in a 1 per cent aqueous solution of the same for one hour. Afterwards fixation was completed by immersion in trichloracetic acid, Bouin's fluid, or Zenker's fluid and the series of sections was stained by iron hematoxylin, Congo red, and light green. By this method some of the turns of the cochlea give very good preparations of the structure of the membrana tectoria. The mitochondria also are visible within osteoblasts, osteoclasts, connective-tissue cells, all epithelial cells, and the sensorial elements.

ANATOMICAL SUBSTRATUM OF THE MEMBRANA TECTORIA.

In spite of numerous investigations many features remain obscure in the histogenesis and structure of the membrana tectoria, in its connections with the adult organ of Corti, and in its extension beyond the sensorial epithelium. Embryologists almost all agree that the membrana tectoria appears in the earliest stages of development of the membranous cochlea before the appearance of the greater and the lesser epithelial ridges and the crista spiralis, as a kind of very thin membrane

on the surface of the somewhat thick epithelial layer covering the interior wall of the ductus cochlearis next to the scala tympani. While the two epithelial ridges and the crista spiralis are developing, the membrane thickens and is in close contact with the surface of their superficial epithelium, which I consider as the generating substratum, the matrix of the membrane of Corti, according to the investigations of most authors (Boettcher 1869, Nuel 1878, Pritchard 1876, Retzius 1884, Denis 1901, Rickenbacher 1901, Held 1909). Others believe that the process of formation extends farther over the surface of Hensen cells and Claudius cells (Hensen 1871, Tafani 1882, Dupuis 1894, Coyne and Cannieu 1895, Czinner and Hammerslag 1898, Vasticar 1909, Prentiss 1913). Previous authors asserted that the membrana tectoria reaches and is attached to the ligamentum spirale (Corti 1851, Claudius 1855, Boettcher 1859, Henle 1866, Loewenberg 1868, Barth 1889). Everyone who has studied this question has recognized that the greater ridge is the most active segment of this substratum; the crista spiralis, at the surface of which the membrane remains fixed in the adult cochlea, is of less significance; indeed, its activity ceases before the stage of complete development of the organ of Corti. A few authors, with Koelliker (1859), assert that the lesser ridge does not take part in the formation of the membrane, and Vernieuwe (1905) and Hardesty (1908, 1915) attribute very little importance to it.

Most investigators concur in regarding the membrana tectoria as a cuticular product of the cytoplasmic apices of the surface epithelial cells (Koelliker 1861, Hensen 1863, Middendorp 1867, Rosenberg 1868, Winiwarter 1870, Gottstein 1870, Pritchard 1876, Nuel 1878, Retzius 1884, Coyne and Cannieu 1895, Denis 1901, Rickenbacher 1901, Vernieuwe 1905, Hardesty 1908, Vasticar 1909, Prentiss 1913); whereas some (Boettcher 1869, Ayers 1891, Czinner and Hammerslag 1898) consider it to be produced by hairs, cilia, or filaments. Held (1909) practically confirms this opinion. I must, therefore, consider the apices of the superficial epithelium which enter into this process of genesis.

THE GREATER AND LESSER EPITHELIAL RIDGES.

On a transverse section of the tympanic wall of the cochlear duct, in the earliest stages before the appearance of the crista spiralis (pig embryo 60 mm.), and later, when the crista spiralis and the two ridges are visible but before any trace of differentiation has taken place in the sensorial elements, the wall of these regions is lined by a rather thick epithelium which was regarded by certain earlier authors (Koelliker 1859, Middendorp 1867, etc.) as formed by superposition of many rows of cells. But Hensen (1863), Boettcher (1869), Baginsky (1886), and other more recent investigators describe it as a simple columnar epithelium. The elongated prismatic cells reach the inferior and superficial part of the layer and their nuclei are situated at various heights. Figure 1, from a new-born dog, shows such a section near the top of the cochlea, with the first indication of the greater ridge (*gr*) and with the future lesser ridge (*lr*) not yet prominent. A superficial mosaic is visible at *mg*, *ml*, on the two segments, but without any differentiation in the sensorial fields. Many rows of nuclei (*n*) are apparent in the segment of the future lesser epithelial ridge.

In figure 2 are displayed the same details in a transverse, slightly oblique section of the second turn of the cochlea in a pig embryo (93.5 mm.). The lesser epithelial ridge is barely indicated (*lr*) by a superficial mosaic (*ml*), of which all the polygons belong to indifferent epithelial cells, although below them are seen three special nuclei (*ns*) separated by a considerable distance from others more deeply situated (*nsu*). This superficial location of the nucleus is the first sign of sensorial differentiation in an epithelial cell. The deep nuclei belong to future supporting cells. The superficial mosaic of the greater ridge is visible (*mg*), and just at its lateral or outer side is a row of five fields, three larger (*ih*) separated by two smaller (*is*), three apices of future inner hair-cells separated by two apices of future inner supporting cells. The outer hair-cells appear later. Toward the axial part of the greater ridge exist three mitotic figures (*mi*) located below the superficial mosaic; they are the last existing traces of the proliferation zone of Baginsky (1886), which is very well marked in earlier stages.

Figure 3 represents a transverse, slightly oblique section of the greater (*gr*) and lesser (*lr*) ridges on the second turn of the cochlea in a new-born dog. N. Van der Stricht (1908) describes many similar figures in his photos 42, 42′, 44, 45. I will emphasize only the details reproduced on a greater scale in figure 4, a section tangential to the surface of the two epithelial ridges. These confirm for the dog the description given by N. Van der Stricht of embryonic bats, as shown in his photo 52 among others.

From the axial towards the outer region of the two ridges in figure 4 are the following:

1. The superficial mosaic of the greater ridge (*mg*), the most lateral polygons of which are differentiated into one row of circular inner sensorial fields, the apices of the inner hair-cells (*ih*) regularly separated by compressed elongated narrow fields, the apices of the inner supporting cells (*is*).
2. A row of small polygons, the apices of the inner pillars (*ip*).
3. A first row of apices of outer hair-cells (*oh′*) separated by the phalanges of the outer pillars (*op*).
4. A second row of apices of outer hair-cells (*oh″*) separated by the phalanges of the first row of Deiters cells (*d′*).
5. A third row of apices of outer hair-cells (*oh‴*) separated by the phalanges of the second row of Deiters cells (*d″*).
6. The apices of the third row of Deiters cells in the form of small polygons (*d‴*) similar to those of the inner pillars.

Figure 5 is from a segment between the second and third turns of the same cochlea and shows identical structures. But there exists a fourth row of outer hair-cells (oh^{iv}) and a fourth row of Deiters cells (d^{iv}) along a very small portion of the cochlea. Retzius (1884) states that the organ of Corti in the rabbit and the dog exhibits a fourth row of outer hair-cells in the superior part of the middle turn and along the largest part of the apical turn. Waldeyer (1872) mentions a fourth row in man and Retzius (1884) confirms this, but adds that the fourth row and even a fifth belong to the upper part of the cochlea and are largely interrupted. I must point out that the outer sensorial fields of the recently differentiated part

of the organ of Corti are much smaller than the older inner fields (*ih*, fig. 4). In figure 5 all the apices of the hair-cells are of the same size and the superficial horseshoes (*hs*), cut from their subjacent cuticular dark plate from which the hairs proceed, are more clearly visible.

If figures 1, 2, 3, 4, and 5 be carefully compared with one another and with other similar epithelial areas in the cochlea duct, it will be observed that the polygons vary in size. In the first stages of development (figs. 1 and 2) they are largest on the surface of the greater epithelial ridge and much smaller on the future lesser thickening, where their size does not exceed that of the apices of the Hensen cells (*mh*). But in more advanced stages the fields of the crista spiralis (*mcr*, fig. 6) and of the Hensen cells become the largest. They do not alter very much on the surface of the greater thickening, although when the organ of Corti is differentiated they extend a little and retain this size more or less until the membrana tectoria becomes detached from its anatomical substratum (fig. 6, *ssp*). But now they (*mg*) manifestly decrease along an inner segment (*min*) of the greater ridge near the vestibular lip (fig. 6). Figure 24 (N. Van der Stricht) shows this detail much better than my figure 6.

Figure 4 shows the alterations already mentioned as undergone by the primitive small polygons (*ml*) in figures 1 and 2 when the sensorial (fig. 4) and supporting fields appear. During the development of the membrana tectoria they remain more or less unchanged, as may be seen in figure 5; but before reaching the stages of the adult organ of Corti, gradual transformations occur at the apices of the inner and outer pillars and in the terminal bars which form the superficial membrana reticulatis, as described by N. Van der Stricht. To recall details discussed by this author I will give figure 7, which shows the apices of all the constituents of an adult organ of Corti in the bat, in order to demonstrate in connection with the fields of the inner supporting cells a fact of some importance, upon which I shall dwell later, when I speak of the terminal bars.

Within each area of the indifferent mosaic of the greater ridge exists a central corpuscle (*cp*), a constituent of the attraction sphere (figs. 1, 2, 4, 6). In reality, on transverse section one sees a diplosome, two granules superposed, of which only one is visible in a tangential section. Generally central, they may become eccentric and even reach the periphery of the field. The corpuscle is surrounded by a small, clear area, the medullary zone of the attraction sphere of E. Van Beneden, which is itself encircled by a darker cortical zone (fig. 4).

The diplosome also exists in the small fields of the indifferent mosaic covering the future lesser ridge (figs. 1 and 2). When differentiation occurs and is completed the diplosome persists within the sensorial fields, where it becomes peripheric, occupying the outer or lateral part of the round apex of the hair-cell. It is always surrounded by a small, clear medullary area (*cp*, fig. 5) beyond the dense, intensively stained circular central plate from which proceed the hairs. This superficial plate is considered by N. Van der Stricht (1908) and Held (1909) as a cuticular product of the cytoplasm. Series of preparations of the cochlea of young cats, fixed by osmic vapors or 1 per cent osmic acid followed by treatment

by other agents, show the presence of innumerable mitochondria and chondriomites throughout the cytoplasm of the sensorial cells. Near the free surface of these elements the mitochondria increase in number and are in very close contact; on the surface they form a plate which is more or less homogeneous, as if the granules were fused together. In successful thin preparations the mitochondrial nature and the granular structure of the superficial plate may be observed. This proves that cuticular formations belonging to the first series mentioned above may be of mitochondrial origin, but in addition it is a striking proof of the mitochondrial nature of the acoustic hairs formed by this plate. F. Spee (1901), Held (1902), and N. Van der Stricht (1908) have described the central corpuscles of the hair-cells, and the last two authors are agreed that these diplosomes do not take part in the formation either of the cuticular plate or the hairs. According to N. Van der Stricht, the superficial central corpuscle of the hair-cells (crista and macula acustica) forms a flagellum; Held (1909) observes two flagella for each diplosome within all the epithelial cells lining the cochlear duct. The superficial central corpuscle shows a flagellum prominent on the surface and on the deep face a flagellum directed into the protoplasm towards the nucleus.

The diplosomes within the irregular polygons of the sustentacular fields of the organ of Corti are repelled into the enlarged axial or inner portion of the inner supporting cells (fig. 4), into the enlarged lateral portion of the phalanges of the outer pillars as observed by N. Van der Stricht and Held. In the phalanges of the first and second rows of Deiters cells (and of the third in case of an additional fourth row) in the new-born dog they are divided into two central corpuscles, one of which reaches the axial segment and the other the lateral segment of the field. At this stage of development (figs. 4 and 5) the central corpuscles are not displaced in the small polygons of the inner pillars, in the third row of Deiters cells, and in the fourth when it exists.

What is more important in elucidation of the anatomical substratum of the membrana tectoria is the appearance of the terminal bars—the system of lines which separates all the polygons, the apices of the cells, of the superficial mosaic, and closes the intercellular spaces. These bars, described for all endothelia and columnar epithelia, represent a denser and superficial portion of the intercellular substance, the chemical composition of which is altered, for it takes up intensively various stains (such as iron hematoxylin) in the same manner as do the central corpuscles and the cuticular superficial plates of the acoustic cells. The size of the terminal bars varies according to the stage of development and the region. Originally thin, they remain thus in the region of the cells of Hensen and of Claudius. They enlarge slightly at the surface of the crista spiralis, but become much thicker on the greater epithelial ridge and between the constituents of the organ of Corti.

My preparations from pig, bat, dog, cat, and mouse enable me fully to confirm the results obtained upon bat (*Vespertilio noctula*) by N. Van der Stricht, who considers the membrana reticularis of the crista and macula acustica and of the organ of Corti as formed exclusively by a gradual enlargement of the terminal bars. In 1876, after fixation of material by silver nitrate which stained the inter-

cellular cement (Kittsubstanz) in black, Lavdowsky expressed the opinion that the membrana reticularis is formed by this metamorphosed substance.

Figure 8, a section tangential to the surface of the crista acustica in a newborn dog, shows a system of thick terminal bars (*tb*) between the smaller polygonal supporting fields (*suf*), each of which presents a central corpuscle and the larger more circular sensorial fields (*sf*), within which a dark central plate and an eccentric central corpuscle (*cp*) are visible.

Figure 9 represents a similar appearance of the crista acustica in an adult bat (*Vespertilio fuscus*). Here the bars (*tb*) are very much enlarged and extend over the greater part of the clear sustentacular fields, leaving uncovered only their central area (*suf*). The more or less circular openings (*suf*) of the membrana reticularis become smaller in figure 10, the crista acustica of an adult white rat, and are narrowest in figure 11, the macula acustica of an adult mouse. The much larger sensorial fields (*sf*) of these last three figures show the central dark cuticular plate from which proceed the hairs traversing these large openings of the membrana reticularis. The power of enlargement and extension of the originally thin terminal bars is fully demonstrated by these four figures, as also is the real origin of the fenestrated membrane derived from them.

As regards the origin of the membrana reticularis of the organ of Corti, figures 4, 5, and 7 are noteworthy. During the earliest stages in the process of development of the membrana tectoria the bars separating sensorial and supporting fields are rather thin, although much thicker than those visible in my preparations at the surface of the cells of Hensen and of Claudius, but they gradually enlarge, chiefly after the membrana tectoria is formed. In the adult organ of Corti one sees (fig. 7) well how the bars have become thicker everywhere and are enlarged most between the first and second rows of outer hair-cells and in such a way that between these two rows, and again between the second and third row, there is to be seen a system of lines alternately thin and thick (*tb''*), but relatively thicker in the latter situation. Finally, along the row of inner hair-cells between two neighboring sensorial fields (fig. 7′, *ih*), there is a small, dark veil hiding the apices of the inner supporting cells. This originates as an extension of the terminal bars, and I was able to see similar figures and superficial veils in the organ of Corti of adult rats and dogs. The development of this veil is another striking proof of capacity for extension over neighboring cells possessed by the terminal bars. It may be recalled here that the existence of a small plate or a prolongation of the head of the inner pillar has been mentioned previously by Retzius (1884) and Held (1902). According to Retzius, who did not recognize the inner supporting fields, it extends between the apices of two inner hair-cells. Held, who terms this prolongation a rostrum (Schnabel) or a bill, describes it as spreading over a small outer zone of the inner supporting cells. I presume that this *rostrum* is a part of the superficial veil which I have described as derived from the terminal bars, but which these two investigators did not recognize.

During the development of the membrana tectoria the terminal bars, as already stated, possess the power to grow and thicken upon the surface of the greater

epithelial ridge and even of the crista spiralis. This property may result in further alterations. At certain places the bars show a tendency to split longitudinally into two parallel lines, the clear space between which is bridged across, as in the case of so many intercellular spaces between epithelial cells. According to N. Van der Stricht (1908), this process of longitudinal splitting of the bars occurs regularly in the course of development of the membrana reticularis covering the crista and macula acusticæ. It also takes place during the formation of the membrana limitans olfactoria (1909). The power of extension of the bars over the apices of neighboring cells I will discuss in a later chapter.

The superficial epithelial mosaic of the cochlear duct has been described by Lavdowsky (1876) and Retzius (1884). Both investigators used silver nitrate as a fixing agent and thus stained the intercellular cement black. Vernieuwe (1905), after staining the terminal bars intensively blue by iron hematoxylin, first recognized the true nature of these elements and their special chemical composition. He observed the indifferent mosaic of the greater ridge, and since the number of fields and the number of nuclei deeply situated are approximately the same, he concluded that all the cells reach the surface, and consequently that this thick epithelium ought to be considered as a simple columnar epithelium. In 1902 the terminal bars on the surface of the adult organ of Corti had already been described by Held; in 1908 N. Van der Stricht and in 1909 Held studied them in the embryonic and adult cochlea on the surface of the indifferent and sensorial epithelium.

CRISTA SPIRALIS, LIMBUS SPIRALIS, HABENULA DENTATA, HABENULA SULCATA.

Former authors, including Huschke (1832) and Corti (1851), mention the existence of two regions in the surface of the crista spiralis. One, lateral—the zona dentata or sulcata, near the vestibular lip of the sulcus spiralis—displays a series of elongated protuberances more or less parallel—the teeth of Huschke, separated by furrows within which Corti had previously noted vestiges of nuclei. Another region, axial, near the attachment of Reissner's membrane, exhibits prominent "warts," "swellings," or papillæ, and may be termed the zona papillaris.

The crista is formed by connective tissue which was regarded by Hensen (1871) as cartilaginous, or as intermediary between cartilage and connective tissue. Gottstein (1870) and Waldeyer (1872) considered it, likewise the teeth, as osteoid substance and calcified. Hensen (1863) and Kölliker (1867) described the teeth as a product between and derived from the superficial epithelial cells, but Boettcher (1869), Waldeyer (1872), Denis (1901), and Vernieuwe (1905) among others, demonstrated that they are formed by a proliferation of the subjacent connective tissue between these elements. What becomes of these epithelial cells during and after this proliferation? Boettcher (1869) and V. Winiwarter (1870) noticed rows of nuclei without cytoplasm within the furrows separating the teeth, and Winiwarter described a kind of superficial mosaic without nuclei. He stated it in the following terms: "Sehr eigenthümlich ist die auf der oberen Fläche des Gehöhrwulstes mit stärkeren Vergrösserungen wahrnehmbare Epithel-Zeichnung, hervorgebracht durch feine, scharf ausgedrückte Contouren ohne Spur von Kernen." It is, of course,

the real mosaic figured and described later by Lavdowsky (1876), Retzius (1884), N. Van der Stricht (1908), and Held (1909). After treatment by silver nitrate, Lavdowsky notices on the limbus spiralis a layer of small endothelial cells devoid of nuclei, more exactly endothelial plates which are quite distinct from the subjacent cells located within the interdental furrows.

On the limbus spiralis of rabbit, cat, and man, Retzius, also using silver nitrate, states that the interdental cells situated within the furrows reach the surface and line by their superficial flat apices the prominences, the teeth, and the warts (Warzen). Hence in adult individuals is formed a complete cell mosaic, continuous with the cell-layer of the Reissner's membrane and of the sulcus spiralis. This statement is confirmed by N. Van der Stricht and Held, who used other fixing agents and stained the terminal bars between the apices of the epithelial cells and found a diplosome within each polygon of the mosaic.

It is to be pointed out that the last three authors, who accurately describe the superficial mosaic and its connections with the teeth and the primitive epithelial cells, do not give exact details of the location of the cell bodies and of the intermediary connective tissue, because they did not examine transverse sections of the crista spiralis at different stages in its development. There is no wonder that in many text-books of histology the description of the superficial elements of the crista is partly erroneous. I will content myself with referring to the Histology of Stöhr translated by Billstein, 1898. On page 380 one reads: "the surface of the limbus is covered by a simple layer of flattenedep ithelial cells." R. Krause, in the Handbuch der vergleichenden und experimentellen Entwickelungslehre der Wirbelthiere, O. Hertwig, 1900, Bd. 1, p. 118, expresses himself in the following terms: "Die anfangs kubischen Zellen werden ganz platt und bilden eine feine, endothelartige Membran, welche hier den Ductus cochlearis begrenzt." In the text-book of Microscopic Anatomy, by E. A. Schäfer, 1912, one reads on page 285 that the cells "are continued as a pavement-epithelium over the limbus." In 1909 Vastiear described very flattened polygonal cells at the surface of the teeth of Huschke.

In order to get a true picture of the structure of the crista spiralis at various stages in its development, it is necessary to compare tangential sections with the vertical, and to follow, step by step, the formation of the teeth of Huschke and the alterations in the epithelial cells. I will distinguish four stages in succession.

1. The first is represented in the second turn of the cochlea from a pig embryo of 93.5 mm. The columnar epithelium is separated from the subjacent connective tissue by a basement-membrane stained blue by Mallory's method and green by light green, and is in continuity with the much thicker epithelium which outlines both ridges. On vertical and somewhat oblique sections this basement-membrane is fenestrated and provided with small openings; small nuclei of connective tissue, cells are incorporated in its thickness, their axis parallel to the surface of the epithelium. Beneath the membrane exists an embryonic areolar connective-tissue consisting of cells the nuclei of which are stained red by Mallory's method or blue by iron hematoxylin, and surrounded by a very small cytoplasmic zone stained faintly red by fuchsin or rosy by Congo red; this zone is in continuity with prolongations

taking up the same stain. But around and between the cells one sees an alveolar system, on optical sections a network of blue (if stained by Mallory's method) or green (if impregnated by light green) collagenous sheets or filaments in continuity with the basement-membrane. Within the spaces of the reticulum sections of the protoplasmic prolongations of the cells are visible. The alveoli, large in the deep layers, become gradually smaller in the neighborhood of the columnar epithelium. The superficial epithelium is represented by a row of prismatic cells, the nuclei of which may be situated at various heights. Each cell contains a single nucleus and the intercellular spaces are closed by the terminal bars already mentioned.

2. The second stage is that of the beginning proliferation of the connective tissue between the epithelial cells. Figure 12, from a photograph of the second turn of the cochlea in a 127.0 mm. pig embryo, shows this process in vertical section. The basement-membrane (*bm*), more or less visible on the right toward the future zona papillaris, has disappeared toward the left near the sulcus spiralis (*cssp*), where the proliferation first begins and is always most advanced. There exist below the epithelium larger alveolar spaces, and the constituents of the membrane with the collagenous elements of the connective tissue extend between the bases of the epithelial cells in the form of dark intercellular filaments. At first sight this intraepithelial connective substance seems to be homogeneous and no transverse sections of bundles are perceptible. Upon careful observation, however, it shows very small spaces within which thin prolongations (*pr*) of connective-tissue cells are detected. Hence it must be recognized that from the first stage of proliferation cell-prolongations and collagenous walls or sheets penetrate between the epithelial elements. Tangential sections of the crista spiralis demonstrate that this proliferation is performed in such a way that the epithelial cells are pressed together in more or less parallel rows along the future zona dentata (fig. 13, *zd*). The axis of the cellular rows (*ep*) is also parallel to the surface of the limbus and is directed from the future vestibular lip toward the zona papillaris. No collagenous substance exists between cells of the same row. Figure 12 proves that neighboring epithelial cells of the greater ridge, which later will cover the sulcus spiralis (*cssp*), participate in this alinement and special arrangement of the epithelial elements. These very small parallel intraepithelial connective sheets (*ic*, fig. 13) represent, of course, the future teeth of Huschke, which in oblique tangential sections (*t*, fig. 14) are clearly in continuity with thicker subepithelial parallel septa of the same nature (*st*, fig. 14). Consequently, it is obvious that this intraepithelial arrangement in the form of teeth of Huschke is in the first place induced by a special and similar disposition of the subepithelial substratum.

3. The distinctive feature of the third stage is that the connective-tissue teeth are more or less as large as the interdental epithelial sheets. Vertical sections of the crista spiralis in a pig embryo of 127 mm. stained by Mallory's method show this fact (fig. 16). Each superficial epithelial cell (*ep*) seems to contain two or three nuclei (owing to the oblique direction of the section) and to be cylindrical; but the red-stained cytoplasm at its free surface is in continuity with a similar small superficial layer (*mcr*), dark in the photograph, covering the intermediary

teeth (*t*) and representing the superficial mosaic, which is never invaded or traversed by the proliferating connective-tissue. The transverse diameter of some cell-bodies is the same at various heights, but in others it is slightly reduced and constricted near the surface.

Between the dark epithelial elements exists a clearer collagenous mass (*t*), deeply stained by aniline blue, within which are noticed darker filaments stained red. These are the prolongations of subjacent connective-tissue cells (*pr*). These connective interepithelial teeth of Huschke extend bodily into the depth (*st*) and are largely in continuity with the subepithelial substratum. Many preparations show this detail more distinctly.

Tangential sections at this stage are very interesting (figs. 17, 18, 19). Figure 17 shows a portion of the third turn of the cochlea in a new-born dog. Above one sees the superficial epithelial mosaic (*mcr*) like a veil formed by elongated polygons, the apices of the epithelial cells, which are separated by darker lines, thin terminal bars stained blue by iron hematoxylin. The long axis of these fields is perpendicular to the axis of the rather dense subjacent bands (*pb*) which represent cytoplasmic epithelial zones deprived of their nuclei because the razor has taken only the superficial segments of the cells. In the lower part of the figure the veil disappears and one notices nuclear bands (*nb*), the section of the deeper segment of the epithelial elements. These bands are more or less parallel in the zona dentata (*zd*), ramified and anastomosed in the form of a network in the zona papillaris (*zp*). Figure 19 shows much better the reticulum of nuclear bands (*nb*) in a pig embryo of 127 mm. The zona dentata is cut more superficially and one sees its mosaic (*mcr*) and a part of the membrana tectoria (*mt'*). Figures 17, 15, and 18 demonstrate also that the neighboring epithelial cells of the greater ridge (*essp*), conspicuous by their larger and darker nucleus, join the epithelial elements of the crista spiralis in the formation of the cell-bands. The connective tissue between the nuclear bands represents the teeth of Huschke and consists of a clear collagenous mass and darker granules (*pr*), the transverse section of the prolongations of subjacent connective cells.

In areas of figure 17 and more clearly in figure 18 one sees other details. A system of regular parallel filaments crosses the teeth (*t*) transversely like intercellular bridges and connects two neighboring nuclear rows (*nb*). There seem to exist as many bridges as there are nuclei in one column. Preparations of pig embryos of 127 and 137 mm. display similar bridges and their regularity proves that they represent real structures. I think that they are thin cytoplasmic membranes persisting between the cells of two neighboring rows after their separation by the proliferating connective tissue. At this third stage of its development the subepithelial parts of the teeth of Huschke are marked on tangential sections by large, thick, dense, parallel, collagenous bundles, within which many cell prolongations are embedded.

4. The final or adult stage is characterized by the fact that the cytoplasmic epithelial sheets are very thin and constricted in their superficial non-nuclear segment, which is in continuity with the persistently intact superficial mosaic; they are much larger at the level of their deep nuclear segment.

The two vertical sections represented in figures 20 and 21 give a true picture of these conditions. The first is from a pig embryo of 190 mm., the second from a young dog of about 4 months. Compared with figure 16, they show that the teeth (*t*) and the interdental epithelial sheets (*ep*) become much longer, as seen in figure 20. The teeth enlarge superficially, and by compression the intermediary cytoplasmic sheets are mechanically reduced to a kind of membrane which remains in direct continuity with the superficial epithelial mosaic (*mcr*). This latter is stained a little darker than the thin cytoplasmic sheets. In the depth the teeth keep their previous size or enlarge very little between the nuclear portions of the sheets, but from there their transverse diameter rapidly increases toward the surface and toward the depth. On the contrary, the epithelial bands become thinner in both these two directions. At their base the teeth with their cell prolongations merge with the subjacent connective-tissue.

Figure 21 shows the different layers of the adult crista spiralis:

1. Superficially the membrana tectoria (*mt*).
2. The cytoplasmic mosaic (*mcr*) beneath the former.
3. The epithelio-connective layer formed by the teeth (*t*) and the interdental epithelial sheets (*ep*).
4. A subepithelial connective layer formed by a collagenous substance, including occasional spaces with stellate cells and a system of canals with cell prolongations (*pr*). These latter are visible in length, in great number reaching the surface of the teeth. The teeth of Huschke are real extensions of this subepithelial layer.
5. A deep layer where the fundamental substance is less abundant and the cells with their prolongations more numerous.
6. A periosteal membrane (*per*), which later will undergo ossification and form a bone lamella separating the crista spiralis from the subjacent nerve-fibers (*ner*).

My description of the connective tissue is for the most part similar to that of Vernieuwe (1905).

Tangential sections of the adult stage of the crista spiralis are represented by figures 22 and 23. The first is from a pig embryo of 190 mm., the second from an adult bat (*Vespertilio fuscus*). They show three different planes in succession. The first and most superficial is the mosaic (*mcr*), consisting of clear polygonal fields separated by rather thin terminal bars (fig. 23), which in figure 22 are split longitudinally and exhibit intercellular bridges. Figure 24 displays a similar veil from the cochlea of a young dog, but within the polygonal fields one sees a dark circular mass, a kind of plate which represents the attraction sphere (*sph*) formed by a central corpuscle (*cp*), a small, clear medullary zone, and a larger dark cortical zone. In the cochlea of the adult mouse I could notice the successive stages of division of this dark layer into two smaller dark plates. It is an unexampled and surprising process in the evolution of the sphere, the function of which in the cell completely ceases after the last mitosis during the earliest stages of development.

A second plane, cut by the razor a little more deeply, shows a system of dark bands, granular and non-nuclear (*pb*), which become nuclear in the still deeper

third plane (*nb*). They are more or less parallel in the zona dentata and anastomosed in the zona papillaris (*zp*, fig. 22). In preparations from the pig embryo they are stained rosy by Congo red, and in those from the bat they are faintly blue from iron hematoxylin, the nuclei being dark blue. The nuclear bands are larger than the cytoplasmic.

The teeth of Huschke (*t*) between these epithelial sheets are clear and homogeneous in figure 22, like the papillæ of the zona papillaris (*zp*). But in figure 23 the teeth are striated from the presence of long granular filaments stained faintly blue, the prolongations of subjacent connective cells. The fundamental substance of the teeth is stained rosy by Congo red.

Transverse compared with tangential sections permit one to conclude that in the adult crista spiralis the interdental epithelial sheets (lamellæ) are much longer than in previous stages and reach a deeper level in the subjacent connective-tissue, but that they remain in direct continuity with the superficial cytoplasmic mosaic, covering entirely the surface of the teeth and the papillæ. Beneath this mosaic their transverse diameter is the smallest, while in their deeper nuclear portion it remains practically the same as in the preceding stage or decreases a little. This elongation and thinning of the sheets is due to a broadening and mechanical pressure of the intermediary teeth.

The reduction in size and in number of nuclei of the epithelial lamellæ is shown much better by the tangential sections than by the transverse. This change, more apparent than real, may be imputed to their elongation in a direction parallel with the axis of the primitive cell and undoubtedly to the wide extension of the sheets during the increase in size of the crista spiralis. But comparing figures 22 and 23 (where long cytoplasmic bands separate two rather small nuclei) with figures 18 and 19 (where the neighboring nuclei are in close contact) it seems to me that during the development of the limbus spiralis an increase of the cytoplasmic mass occurs and can not be denied.

This consideration brings me to the important question of study of the epithelial lamellæ of the crista spiralis; all authors describing vertical sections of this admit the existence of separated cells, the primitive epithelial cells. Indeed, figures 12 and 13 prove that during the first and second stages this view is correct, and figures 16, 20, and 21 seem to confirm it for the third and fourth stages. All sections tangential to the surface of the crista, however, show different figures. At the third stage (figs. 17, 18, 19) no boundaries between cell-areas can be detected either in the cytoplasmic or in the nuclear bands where the nuclei are very closely pressed together. This statement is also true for the fourth stage (figs. 22 and 23). Consequently these epithelial sheets must be considered as real syncytial masses formed by fused epithelial cells, which are separated only during the first and second stages of their development. This fusion is brought about by a mechanical factor, the compression from the broadening teeth. This multinuclear syncytium is unexampled in other organs, while here the primitive apices and central corpuscles of the epithelial cells persist intact, the boundaries being marked by the terminal bars. Retzius (1884) and N. Van der Stricht (1908) refer to similar figures, but

the former author, though his illustrations show undivided nuclear cytoplasmic masses, speaks of "interdental cells;" the second mentions "nuclear bands." Neither describes the syncytial nature of these formations.

As a matter of fact, in many preparations some exceptions to this rule may be found with spaces between the neighboring cells. I do not refer, of course, to figure 20, where the razor cut the teeth vertically just at the edge of the vestibular lip and struck the epithelial cells of the subjacent sulcus spiralis, an unchanged columnar epithelium (*essp*); I refer to figures which may be observed in the middle of the limbus. Their existence proves that when the mechanical pressure is weak fusion does not occur, or when it diminishes in adult stages boundaries may reappear.

Do the numerous nuclei of this special kind of syncytium represent elements of the primitive isolated cells? Or are new nuclei formed at the time of fusion of the cell-bodies or afterward? As a matter of fact, after the first stage described above, no mitoses occur within the epithelial cells. On the other hand, some preparations seem to confirm the idea that occasionally nuclei undergo nuclear amitosis, increasing in size and elongation, and exhibiting direct division into two smaller daughter nuclei by a process of constriction.

Retzius was able to investigate this question. His figures represent the superficial mosaic with terminal bars stained black by silver nitrate; below each polygon he notices a single nucleus, hence he asserts that there exist as many apices of cells as nuclei, although near the vestibular lip some fields contain two nuclei which he explains as due to the fact that the supplementary elements belong to the sulcus spiralis. But by a careful examination of the figures of Retzius I can sometimes count three nuclei beneath two superficial fields at a short distance from the vestibular lip (his figure 1, plate 24) and also in the zona papillaris.

If, in preparations similar to that displayed in figure 17, I compute the number of superficial fields along one row of polygons of the zona dentata and the number of nuclei of one nuclear band, I find that I obtain as a rule the same number (about 12); but there are some exceptions where there are one or two nuclei more than fields. I am inclined, consequently, to believe that some occasional nuclei of the primitive epithelial cells undergo the process of amitosis during the second or third stage of development of the crista spiralis.

Finally, I think that it is worth while to emphasize the fact that furrows mentioned by many authors between the prominences of the teeth, within which (according to v. Winiwarter and others) nuclei or remains of epithelial cells are located, do not exist at all. There are large interdental spaces completely filled by the epithelial syncytial lamellæ. In the adult organ the deep portion of these spaces is broad, but the superficial gradually becomes very narrow at the level where the cytoplasm is in continuity with the superficial mosaic. Superficial pits between the teeth are either exceedingly small or are lacking; if they do exist they are covered by only a part of the mosaic. The fused epithelial cell-bodies are embedded between the teeth in such manner that I can not agree with Retzius (1884, p. 345) when (in describing these conditions of the adult man) he states: "Diese Epithelzellen welche sich beim Embryo als eine cylinderzellen Schicht anlegten sind also noch beim

Erwachsenden als Cylinderzellen erhalten, nur sind sie reihenweise von einander durch die von unten her emporwachsenden Vorsprünge getrennt worden."

Retzius adds that these cells are actually so adherent as to remain attached to the membrana Corti when it is torn away from the underlying tissues and that even after maceration they still remain connected with the membrane! Such incidents may occur only when the first or second embryonic stage persists. In this respect I am able to state that large areas of the zona papillaris from cats 1 to 11 days old are covered by a cubical epithelium free from connective-tissue, while other parts of the same region and the zona dentata show the structure of the adult stage. According to Gottstein (1870) and Waldeyer (1872) half the surface of the crista spiralis, next to the attachment of Reissner's membrane, remains covered by a continuous layer of epithelial cells. Of course, such elements may become detached by maceration or by stripping off the membrana tectoria.

DEVELOPMENT OF THE MEMBRANA TECTORIA.

ON THE SURFACE OF THE GREATER EPITHELIAL RIDGE.

The process of genesis of the membrana tectoria is conspicuous mainly at the most active portion of its anatomical substratum—that is to say, at the surface of the greater epithelial thickening. Tangential sections must be obtained, since they exhibit the best figures, as demonstrated by figures 25 and 26. One observes the superficial mosaic (*mg*) removed by the razor from small areas (*mt'*), where the recently formed part of the membrana tectoria is visible. At *mt'* exists a kind of pale mosaic of another nature, reproducing that of the subjacent anatomical substratum. The terminal bars are replaced by larger dense lines and the apices of the cells by paler, more circular areas. The lines exactly overlie the system of terminal bars which is cut off, and their substance is not only in close contact with the bars, but is in continuity with them at the periphery of the small islands. The pale, more fluid substances in the mazes of this network overlie the polygons of the mosaic, the cytoplasmic apices of the epithelial cells, and are also in continuity with them. Hence the compact lines must be considered as produced by the bars and the content of the mazes by the superficial cytoplasm.

By Mallory's method the lines are stained blue, the bars red; by the use of iron hematoxylin and Congo red the former are rosy, the latter dark blue; after iron hematoxylin and light green the first are intensely green and the second dark bluish. Consequently, the chemical composition of the line must be regarded as different from that of their generating substratum.

The cochlea of a pig embryo of 93.5 mm. fixed by the uranium nitrate method of Ramon y Cajal shows in some places (fig. 27) the terminal bars (*tb*) and the dense part (*tb'*) of the superficial membrana tectoria stained black. The compact lines (*tb'*) are thinner than the bars and situated in a different plane; hence they are not quite in focus. The result of this treatment affords another striking proof of the real origin of the lines and of a chemical composition more or less similar to that of the bars. The lines (*mt'*, figs. 25 and 26) are larger than the bars and at first sight

seem to be homogenous and structureless; but on careful examination (*mt'*, figs. 25', 26') they look double, as if split longitudinally into two parallel thin lines severed by a clear space which is at times bridged across, the bridges being immersed in a kind of intercellular substance.

A tangential section through all the layers of the membrana tectoria (*mtg*) over a large extent (fig. 28) proves that the same structures are visible everywhere, the mazes of this kind of network becoming a little smaller toward the surface of the membrane (on the right side of the figure) than in the vicinity of its inferior side, next to the mosaic of the greater ridge. Nowhere can there be seen transverse sections of filaments or of fibrils. Hence the reticulum in figure 28, and *mt'*, figures 25 and 26, must be considered as the optical section of a system of walls, of membranes surrounding cylindrical or prismatic tubules filled with a pale fluid. In other words, the membrana tectoria is formed by a system of cylinders or prisms consisting of a dense outer wall derived from the terminal bars and of a contained portion, the more fluid part, derived from the cytoplasmic apices of the epithelial cells.

This view is confirmed by vertical sections through the greater epithelial ridge, as represented by figures 29, 30, and 31, taken from the cochlea of a pig embryo of 95 mm. These show the cylinders or prisms lengthwise as double lines (*cy*) and cut across as circular or polygonal fields (*cy'*). Their transverse sections in figures 30 and 31 are quite similar to those in figures 28, but the longitudinal sections (*cy*) demonstrate better that between the cylinders exists an interprismatic clear substance with delicate structures, within which elements like bridges can be noticed. Figure 29 shows most clearly that the young membrana tectoria consists of a basal clear layer and one more superficial, darker and more compact, the walls of the cylinders being denser near the surface and their diameters being a little smaller, with an intermediary substance less abundant. Finally, the base of each prism is obviously in continuity with one of the slightly prominent apices of the epithelial cells; but these vertical sections can give no sure knowledge concerning the origin of the constituents of the membrana tectoria. Therefore, tangential sections are needed.

We shall see that the interprismatic substance undoubtedly exists in the membrane of adults; hence it is not an artificial product, the result of shrinkage. It is derived from the terminal bars which may split longitudinally into two thinner parts connected together by short bridges. The primitive bars close the subjacent intercellular spaces and separate the intercellular cement from the interprismatic substance, which must therefore be considered as derived from the bars themselves.

Some authors, Coyne and Cannieu (1895), Hardesty (1908), Held (1909), and Prentiss (1913), have drawn and described figures similar to those in my figures 25, 26, *mt'*, and also regard them as representing the first stage of the developing membrana tectoria. Hardesty and Held consider these figures as a network of filaments derived from the superficial cytoplasm of the epithelial cells and forming the fibers of the adult membrane. Hardesty (1915, p. 60) states:

"In the production of the tectorial membrane each cell of the greater epithelial ridge may contribute an average of 25 fibrils to the membrane. Each fibril seems to show a

slightly elongated enlargement at its junction with its cell. In the region of the immediate surface of the ridge the interfibrillar matrix does not appear as abundant or so completely produced as in the older body of the membrane."

In many figures Hardesty and Prentiss represent the bars, but do not describe them.

Held computed 33 to 38 fibrils per each 100μ of the surface of the greater ridge. They are stained red and visible as red granules on sections tangential to the superficial mosaic, each polygon displaying some of them (his figure 3, guinea-pig). Nowhere else does this author mention transverse sections of these elements in the developed membrane, although occasional figures seem to show them. He describes the terminal bars everywhere, but does not attribute to them any importance in the genesis of the membrane. However, investigating the origin of the cupula of the surface of the macula acustica in the rabbit, he states that the reticulum, representing the first stage of development of the cupula, remains attached to the apices of the sustentacular cells by delicate filaments, "wobei sie oft nicht in mitten der Zellfläche, sondern mehr einer Schlussleiste zu sich anheften" (p. 265). He mentions further, beneath the cupula, special filaments in connection with the surface of the sensorial epithelium, and adds: "merkwürdigerweise sind diese besonderen Fäserchen mit den Schlussleisten seitlich verbunden, oft in reicher Zahl von diesen abgehend, welche eine Haarzelle umgreifen." Finally, referring to the filaments of the tectorial membrane in the hen, the chicken, and the pigeon, he states that they "an den freien Flächen der Stützzellen und oft dicht an den Schlussleisten sich anheften" (p. 275). In spite of these statements, Held accords no importance to the terminal bars in the development of the membrana tectoria.

Held gives further details of the anatomical substratum of the membrane. In the rabbit he observes a special homogeneous border on the surface of the epithelial cells, within which the diplosomes and the terminal bars are inclosed. It is his "Randsaum" and undoubtedly my superficial mosaic. But above it appears another very thin striated border, his "Decksaum," and on page 203 he states: "Die Vorstellungen die ich nur auf Grund dieser Beobachtungen gebildet habe, ist, dass der durch den Rand- und Decksaum ausgezeichnete Epithelbezirk die erste Bildungszone der Cortischen Membran ist."

Figure 32 represents a portion of the membrana tectoria and the superficial mosaic of the subjacent greater ridge in the vicinity of the future sulcus spiralis (*ssp*) partially free and detached from the membrane. It displays some structures very interesting in relation to the fibrillar origin of the membrana tectoria as described by Hardesty and Held. While some fields of the mosaic are clear (*f*), others are covered by a dark veil (*f'*) or a granular veil stained like the bars. Indeed, many preparations prove that these latter are able to enlarge and extend over the neighboring polygons. But some structures, real filaments (*f''*) derived from the bars, incline and join together over the polygons more superficially where they (the said structures) generate the dense part of the cylinders. If these fibers persist in the walls of the prisms one would have to recognize the fibrillar structure of the walls, but my preparations do not allow me to ascertain with certainty if this be so.

The same figure shows also the extent of shrinkage often produced by fixing agents through artificial distention of the more fluid part of the recently formed layer (*mt'*) of the membrana tectoria.

Coyne and Cannieu describe the membrana Corti as by no means formed by a homogeneous clear substance with dense fibrils, but by membranes or sheets ("cloisons") of a special nature circumscribing polygonal cavities which form a network in perpendicular sections. "The surfaces of junction of these membranes thickened at the angles of the reticulum" (p. 280) represent the alleged fibers; and on page 285 they add: "these sheets circumscribe polygonal cavities which gradually become narrower from the organ of Corti toward the prominence of Huschke."

My results upon the development of the membrana tectoria are chiefly comparable with those of Prentiss. On page 442 this author states:

> "To sum up the development of the membrana previous to fetuses of 15.0 cm., we may say that it is a cuticular organ with a definite though irregularly chambered structure which is secreted between, and at the ends of the cells composing the basal epithelium of the cochlea."

In his conclusion Prentiss adds:

> "In sections through the axis of the cochlea the membrana has a striated or lamellated appearance. . . . In sections perpendicular to the lamellæ the structure of the membrana is that of a reticulum with thickenings at the angles of the meshes. It is therefore neither lamellar nor reticular but a chambered structure or 'honeycomb' of hollow tapering cuticular tubes or chambers normally filled with a fluid resembling the endolymph. The bases of chambers during development rest between the ends of the epithelial cells."

The main difference between the results of Prentiss and my own is that according to my investigations there may exist an interprismatic substance among the chambers, and that the walls of the cylinders are produced by the terminal bars which were not recognized by Prentiss, while their content alone is formed by the cytoplasmic apices of the cells.

ON THE SURFACE OF THE LESSER EPITHELIAL RIDGE.

Figure 28 displays a section tangential to the surface of the organ of Corti, the neighboring greater ridge and a segment of the crista spiralis between the second and third turn of the cochlear duct in a new-born dog. *Ih* and *oh'*, *oh''*, *oh'''* show respectively the row of inner hair-cells and the three rows of outer hair-cells. Two hair-cells, oh^{IV}, belong to an interrupted fourth row of outer sensorial elements. This segment of the section and another (*mg*, the superficial mosaic of the greater ridge) are not in focus; therefore they are blurred, but the structures of the membrana tectoria are plainly visible on the right of the figure. In continuity with the rows of outer hair-cells one sees three different, more superficial planes *s'*, *s''*, *s'''*. The first (*s'*) displays horseshoe-like elements, the bases of the acoustic hairs; they occupy more or less the center of clear areas separated by a system of darker, thick, longitudinal lines (*l*) and of thinner transverse lines (*l'*). Some of these lines are double and short bridges connect the two halves. The clear areas undoubtedly correspond to the sensorial round fields of the outer hair-cells and overlie their

apices and the pale fluid must be considered as derived from this cytoplasmic substratum. The longitudinal, thick, irregular lines overlie the apices of the supporting fields better visible in figure 5, *op*, *d'*, *d''*, *d'''*, where the terminal bars between two neighboring hair-cells of the same row seem to form a single thick line; while the thinner transverse lines overlie those bars, also irregular, visible between the different rows. Hence the longitudinal and transverse lines of the first superficial plane (*s'*) should be considered as derived from the subjacent terminal bars—that is to say, from the real membrana reticularis of the organ of Corti.

I believe, consequently, that I am justified in drawing the conclusion that the membrana tectoria is formed at the surface of the organ of Corti by the same process as that observed on the surface of the greater ridge. The differences in appearance of the figures are induced by corresponding differences in the anatomical substratum.

The second more superficial plane is also very instructive. It consists of two fields (*s''*) identical in size with those of the preceding (*s'*). Their longitudinal lines (*l*) are undoubtedly double and the two neighboring halves are connected by short bridges. The transverse lines can not be recognized and a delicate pale network is visible within the clear areas. The structures of this plane resemble those of the third superficial plane (*s'''*), where the longitudinal lines have also disappeared, and the constituents are the same as those of the neighboring membrana tectoria belonging to the greater ridge (*mtg*).

What is the significance of the pale network appearing within the clear spaces of the second plane (*s''*)? Two different explanations may be given. The network represents the most superficial portion of the membrane formed in the early stages of its development, before the appearance of the hair-cells, when the subjacent mosaic is formed by undifferentiated polygons (figs. 1, 2, *ml*). Figure 33, from a pig embryo of 150 mm., shows the membrana tectoria recently produced (*mt'*) on the surface of the second and third rows of Deiters cells (*d''*, *d'''*) and the third row of outer hair-cells (*oh'''*). One sees clearly (*mt'*) a series of pale round fields surrounded by very thick bands which, with similar lines around smaller areas, reproduce more or less the subjacent membrana reticularis of the lateral part of the organ of Corti. This figure proves that in the course of development the structures of the tectorial membrane change here greatly, as do those of the subjacent membrana reticularis, and that the differences between its first (*mt*) and its later-formed layers (*mt'*) become gradually more pronounced. I am thus induced to accept this first explanation.

A second explanation would bring me to consider this delicate reticulum as derived from the bars in such way that the dense part of the membrana tectoria directly formed by them and covering them may grow and extend over the apices of neighboring cells and there give rise to structures like those on the crista spiralis. I will refer to this question later.

Held (1909) describes a system of thick fibers derived from the apices of the sustentacular cells of the organ of Corti and chiefly from the first and second rows of Deiters cells; these filaments he terms "Haftfasern der Lamina reticularis

externa," adhering or attached fibers. They take origin from the phalanges by two limbs:

"Ein innere Schenkel ist von einem aüsseren bei diesem zweispältigen Ursprung der Haftfasern der I und II D. Zellen zu unterscheiden, von denen sich jeder aus einer Summe von Fibrillen sammelt, welche auf die beiden entgegengesetzten und breiteren Ecken der Phalangen-platten hauptsächlich verteilt sind, wenn auch eine geringe Anzahl auf dem schmäleren Mitteil stehen kann" (p. 221).

The double lines or double filaments of Held's figure 15 and the coarse fibers in his figure 16 are undoubtedly the same elements as my lines *l*, figure 29, which I regard not as fibrillar, but as bands or walls, homogeneous like the subjacent membrana reticularis.

It may be pointed out that, with N. Van der Stricht, I consider the membrana reticularis of the organ of Corti as fenestrated and constituted by a system of enlarged terminal bars separating openings within which the apices of the sensorial and of the supporting cells are located. Held (1904) and others describe the apices of the Deiters cells as a constituent of this membrane.

The membrana tectoria of the kitten deserves special mention. Fixed by osmic acid and Bouin's fluid or by trichloracetic acid it shows on its lateral edge a regular series of coarse filaments, the thickness of which equals the diameter of the apices of the third row of Deiters cells. Upon each of these apices is adherent a thick, solid, long cylinder, a real cramp (un crampon), even where the entirely free part of the membrana tectoria is detached from the organ of Corti. I am not able to determine if these attachments persist in adult animals. The *cramps* mark clearly the lateral boundaries of the membrane and of its anatomical substratum. I have not, so far, had the opportunity of investigating the earliest stages of their development, since I have at present no embryonic cat material available. It seems to me that Retzius (1884) observed these elements in the cochlea of the cat, since he described, in the *Randfasernetz*, "glänzenden parallel neben einander von innen unten nach aussen oben verlaufende Fasern, welche sich am Ausserrande zur einem Randstrang sammeln."

ON THE SURFACE OF THE CRISTA SPIRALIS.

All authors agree that the crista spiralis takes only a slight part in the development of the membrana tectoria. According to my investigations, the alterations of its superficial mosaic in the earliest stages of its activity are essentially the same as those described for the greater epithelial ridge, although thinner terminal bars and larger cytoplasmic fields result in some differences. Figure 28 shows that the most superficial layer of the tectorial membrane, first developed and visible on the right of the figure (*mtcr*), is formed by a system of larger fields and thinner intermediary membranes than those belonging to the neighboring segment (*mtg*) of the greater ridge. But in a layer a little deeper and more to the left the wide areas disappear, because a kind of delicate network covers them. Finally, still more to the left, the membrana tectoria of the crista spiralis exhibits the same structures as those of the greater epithelial thickening.

Figures 19 and 34, from a pig embryo of 127 mm., illustrate better the appearance of these formations. The two tangential sections are a little oblique and involve four successive planes. The first belongs to the nuclear and cytoplasmic layer of the zona papillaris (*zp*); the second and more superficial shows the superficial mosaic (*mcr*) of the zona dentata; the third exhibits wider areas (*mt'*) enlarged by the fixing agents. Some of these areas contain only homogeneous clear fluid, obviously a product of the superficial cytoplasm; they are separated by thick, coarse lines as dark in the figures as the much thinner lines belonging to the subjacent mosaic (*mcr*), the real terminal bars, although in my preparations they are but faintly stained by Congo red. This dense part of the tectorial membrane more recently formed (*mt'*) is derived from the bars and is in direct continuity with a delicate secondary network of the same nature (and therefore of the same origin) covering some of these clear areas. Finally, a fourth quite superficial plane (*mt*) represents the older part of the membrana tectoria and its constituent structures, but is more compact and reminds one of the structures in the membrane produced by the greater ridge.

The vertical sections, figures 14, 20, and 21, illustrate also the fact that the tectoria membrane (*mt*) on the surface of the crista spiralis is formed by a dense part, the uninterrupted walls between small cavities or chambers filled by a clear fluid. On the right of figure 20 the dense part is marked by long lines which are in continuity with similar constituents or cylinders belonging to the free membrane overlying the sulcus spiralis.

The features just described force me to conclude that at this stage of its development the membrana tectoria of the crista spiralis is formed by a system of chambers or cylinders with a fluid content derived from the superficial cytoplasm of the epithelial cells, and by denser walls produced at least in part by the terminal bars. Indeed, as already stated above, during the development of the membrana olfactoria limitans, of the membrana reticularis of the crista and macula acusticæ, and of the membrane covering the apices of the inner supporting cells in the organ of Corti, the substance of the bars extends over the neighboring fields and may form a kind of delicate network which gives rise to secondary structures of the same nature and of the same chemical composition as those of the bars.

In the case of the developing tectorial membrane the primitive bars generate on their surface a coarse primary network of a different chemical composition, the large meshes of which contain a secondary, more delicate reticulum of the same nature and of the same chemical composition. This secondary network is derived at least in part from the primary, perhaps in part also from similar cytoplasmic structures. To what extent the apices of the epithelial cells take part in the genesis of this delicate reticulum I am no more able to determine than Alice Thing has been (1917) in regard to a similar secondary network generating the fundamental substance of the zona pellucida in the turtle.

Many series of preparations of adult and embryonic stages showing features similar to those of figures 14, 19, 34, 20, and 21, prove that a great number of cylinders of the free membrana tectoria reach and are connected with one cytoplasmic

polygon of the superficial mosaic covering the crista spiralis. Each of them is for the most part derived from one polygon of the mosaic of the greater ridge, but also somewhat from a mesh of the secondary network covering one polygon of the crista spiralis, which (according to all investigators) represents the least active segment of the anatomical substratum. But it is impossible to give even an approximate estimate of the exact parts played by these two substrata.

I have been able to describe the genesis of an interprismatic substance between the prisms produced by the greater ridge, but the genesis of similar spaces between the portions of cylinders formed by the crista spiralis and by the membrana reticularis of the organ of Corti is still obscure. If further investigation should confirm the existence of this kind of cement for the first segment and the lack of it in the other two segments, this distinctive feature might enable other investigators to determine accurately the parts of the prisms derived from each of the three segments of the anatomical substratum.

STRUCTURE OF THE ADULT MEMBRANA TECTORIA.

Most authors distinguish three segments in the adult tectorial membrane: a thin, innermost axial segment covering the crista spiralis; an outermost lateral, derived from the lesser ridge; a thick middle segment produced by the greater ridge according to the investigations of: Henle (1866), Löwenberg (1868), Boettcher (1869), v. Winiwarter (1870), Gottstein (1870), Hensen (1871), Lavdowsky (1876), Nuel (1878), Retzius (1884), Barth (1889), Dupuis (1894), Coyne and Cannieu (1895), Held (1909), and Prentiss (1913).

The middle segment is striated and the striations incline from the vestibular lip toward the organ of Corti, the inclination being due to the existence of fibers or of fibrils as stated by Hensen (1863), Henle (1866), Boettcher (1869), v. Winiwarter (1870), Lavdowsky (1876), Nuel (1878), Tafani (1882), Retzius (1884), Ferré (1885), Ranvier (1889), Barth (1889), Ayers (1891), Dupuis (1894), Hardesty (1908), Held (1909), and Vasticar (1909). According to Coyne and Cannieu (1895), Shambaugh (1907), and Prentiss (1913), they are due to the presence of lamellæ. Most authors agree upon the presence of a homogeneous fluid between these constituents, though Retzius denies it.

The inner segment is described by Henle (1866), Boettcher (1869), v. Winiwarter (1870), Hensen (1871), and Nuel (1878) as a more or less homogeneous but fenestrated membrane provided with openings. Gottstein (1870) and Lavdowsky (1876) regard it as structureless and without openings. Retzius (1884), Barth (1889), and Dupuis (1894) consider it as formed by thin radiating fibrils which according to Held are collected in bundles separating openings. The outer segment, as stated by Henle (1866), Boettcher (1869), v. Winiwarter (1870), Gottstein (1870), Nuel (1878), Retzius (1884), Dupuis (1894), and Held (1909), consists of a network of anastomosed fibrils, filaments, or hyalin bands. Hardesty (1908) describes an accessory tectorial membrane as formed by two sets of fibers crossing at an acute angle.

Prentiss (1913) subdivides the membrana tectoria into the following zones:

1. A thin, structureless zone of the inner portion of the labium vestibulare.
2. A second thicker zone of flattened horizontal chambers over the outer portion of the labium vestibulare.
3. A still thicker zone of chambers curving downward and outward, unattached, over the sulcus spiralis.
4. An outer zone thickest in the upper turn, with chambers trending downward, outward then inward, largely attached to the cells of the spiral organ and probably normally and wholly attached in this manner.

Many investigators describe a superficial layer in some regions of the membrane, the superficial network of filaments, *Fadennetz* of Löwenberg, who mentions its existence throughout the two-thirds of the outer segment. Its existence is confirmed by Hensen (1871), Boettcher (1872), Retzius (1884), Dupuis (1894), Spee (1901), and Held (1909) as extending farther towards the inner segment. Spee and Held recognize that it reaches the attachment of Reissner's membrane and forms the inner zone of the tectorial membrane.

According to the process of histogenesis, the adult membrane is formed throughout its entire thickness and breadth by a system of cylinders or prisms separated by a pale and homogeneous fluid which seems to be lacking in the most superficial and densest layer. Figure 35, from an adult rat, represents a tangential section of the outer segment (*mtl*) and the greater part of the middle segment (*mtg*). At first sight the figure shows a system of double lines, the longitudinal axial section of the cylinders (*cy*). The intraprismatic liquid seems to be a little darker than the more abundant interprismatic fluid. One sees, moreover, other single, often darker lines, tangential sections of cylinders (*cy''*). At the ends of some obliquely cut cylinders the double lines join together and are in continuity with a single line. On the left of the outer segment (*mtl*) and in the middle segment (*cy'*) the prisms are pressed together and their transverse section is visible in the form of small polygons, between which the interprismatic substance seems to be lacking.

In my preparation the segment of the membrana tectoria photographed (fig. 35) is in continuity with its inner segment on the surface of the crista spiralis. This axial portion exhibits the same structures, but the prisms are in closer contact, while the interprismatic fluid is less abundant.

The superficial network of Löwenberg and similar structures of the outer and inner segments are represented, according to my preparations, by more compact parts of the membrane, the interprismatic substance of which is less abundant or lacking, so that a real chambered structure (Coyne and Cannieu, Prentiss), or, in tangential sections, a kind of network appears; but the walls of the chambers or the filaments of the reticulum represent the transverse section of the membranes of neighboring cylinders. Figure 35 displays these longitudinal prisms on the right side of the outer segment, while their apices are cut transversely, pressed together, on the left.

Figure 23, from an adult bat, exhibits a small portion of the middle segment of the tectorial membrane within which at least three similar longitudinal sections (*cy*) of the cylinders and some obliquely tangential (*cy''*) can be noticed.

In addition to these two figures, 23 and 35, of adult stages, I give another, similar, figure 36 and 36′, from a young dog, and one, 37, from a pig embryo 150 mm., with similar structures, longitudinal (*cy*), obliquely tangential (*cy″*), and transverse sections (*cy′*) of prisms. They are larger and the intraprismatic and interprismatic fluid is more abundant. As a matter of fact, in some instances in these figures, instead of prisms with fluid content, there seem to be present transverse sections of solid filaments, and in transverse sections (*cy′*) darker granules like sections of fibers within the walls of the cylinders. Further investigation is wanted to decide if these elements correspond either to real structures or to products of the shrinkage induced by fixing agents, and if some delicate fibrils or bridges may persist within the pale intraprismatic or interprismatic fluid.

From this description I must conclude that the adult membrana tectoria is formed throughout its extent by prisms or cylinders consisting of an outer dense membrane or wall and of an axial clear fluid. They are separated by a more fluid intermediary substance, which may be lacking in some places upon the surface of the middle segment, but chiefly of the other segments, while a wall common to two neighboring prisms separates their pale content. This superficial layer is the densest and formed earliest in the course of development. Fixing agents, by reason of the consequent shrinkage, seem to have a great influence on these structures and induce many alterations.

The results of these investigations permit me to emphasize the analogy of structure and origin of the membrana tectoria and enamel of teeth. Both organs are cuticular formations derived from a columnar epithelium, and their immediate anatomical substratum is represented in the case of the former by the superficial mosaic of the cells, and in the latter instance by a basal mosaic, the cytoplasmic polygons of which are also separated by terminal bars according to the investigations of Cohn (1897). Both are formed by a system of prisms, each produced from one cell. The axial, more fluid part of the prisms of the tectorial membrane is derived from the cytoplasm and the outer denser wall from the terminal bars, which at the same time give rise to an interprismatic fluid. The solid calcified prisms of the enamel are produced by the cytoplasmic base of the ameloblasts, while the part taken by the terminal bars in the origin of the calcified cement has not thus far been demonstrated. It should be pointed out that one epithelial cell of the crista spiralis, and perhaps of the organ of Corti, may produce or be in connection with many cylinders.

After development the enamel becomes completely detached from its embryological substratum. The membrana tectoria, on the other hand, while becoming free in greater part, keeps its primitive connections with the crista spiralis, the least active part of its anatomical substratum.

These results concerning the genesis of the tectorial membrane are of general biological interest, for they prove the importance of the intercellular substance, the terminal bars, in the origin of special constituents of organs considered as of cuticular nature, now established for the membrana reticulares of sensorial organs. In this respect the intimate structure, fibrillar or reticular, of the prisms or of some segments of the membrana tectoria must be regarded as of secondary interest.

The present investigation has been carried out in the anatomical laboratory of the medical school of the Western Reserve University, Cleveland, Ohio. It is an agreeable duty to me to express my very sincere thanks to this university and to the staff of the anatomical department, in which I received gracious hospitality for nearly two years and was allowed to continue my scientific researches. My best thanks go mainly out to Professor Dr. T. Wingate Todd, who put at my disposal all the material, the reagents, and the instruments needed for my investigation. Owing to his kindness, to his ability and assistance, I was able to perform this work. I am very happy to pay to him the tribute of my deepest and most heartfelt gratitude. I also thank very much Mr. G. G. Marshall, who supplied me with a very interesting collection of bats.

BIBLIOGRAPHY.

Ayers, H., 1891. Die Membrana tectoria—was sie ist, und die Membrana basilaris—was sie verrichtet. Anat. Anz., vi, 219.

———, 1898. On the membrana basilaris, the membrana tectoria and the nerve endings in the human ear. Zool. Bull., i, 275–278.

Baginsky, B., 1886. Zur Entwicklung der Gehörschnecke. Arch. f. mikr. Anat., xxviii, 14–37.

Barth, A., 1889. Beitrag zur Anatomie der Schnecke. Anat. Anz., iv, 620–624.

Boettcher, A., 1859. Weitere Beiträge zur Anatomie der Schnecke. Virchow Arch. f. path. Anat., xvii, 243–281.

———, 1869. Ueber Entwickelung und Bau des Gehörlabyrinths nach Untersuchungen an Säugethieren. Dresden.

———, 1872. Kritische Bemerkungen und neue Beiträge zur Literatur des Gehörlabyrinths. Dorpat.

Cannieu, A., 1904. L'oreille interne. *In* Traité d' anatomie humaine de Poirier et Charpy. T. 5, f. 2. Paris.

Claudius, M., 1855. Bemerkungen über den Bau der häutigen Spiralleiste der Schnecke. Ztschr. f. wiss. Zool., vii, 154–161.

Cohn, Th., 1894. Ueber Intercellularlücken und Kittsubstanz. Wiesbaden. (Inaug.-Diss. Würzburg.)

———, 1895. Ueber Intercellularlücken und Kittsubstanz. Anat. Hefte, 1. Abt., v, Heft 15, 295–333.

———, 1897. Ueber epitheliale Schlussleisten an embryonalen und ausgebildeten Geweben. Verh. d. physik.-med. Gesellsch. zu Würzburg, n. F., xxxi, 171–200.

Corti, Alphonse, 1851. Recherches sur l'organe de l'ouïe des mammifères. Première partie. Ztschr. f. wiss. Zool., iii, 109–169.

Coÿne et Cannieu, 1895. Contribution à l'étude de la membrane de Corti. Journ. de l'Anat. et de la Physiol., xxxi, 261–287.

Czinner, H. I., und V. Hammerschlag, 1898. Beitrag zur Entwicklungsgeschichte der Corti'schen Membran. Arch. f. Ohrenh., xliv, 50–88.

Deiters, O., 1860. Untersuchung über die Lamina spiralis membranacea. Bonn.

Denis, P., 1901. Recherches sur le développement de l'oreille interne chez les mammifères. (Vespertilio murinus.) Arch. de Biol., xviii, 377–493.

Dubreuil, G., et Cl. Regaud, 1908. Sur les productions exoplastiques des cellules folliculeuses de l'ovaire chez la lapine. Verh. d. Anat. Gesellsch., Berlin, 152–156.

Dupuis, A., 1894. Die Corti'sche Membran. Anat. Hefte 1, Abt. iii, Heft 10, 447–508.

Duval, M., 1897. Traité d'histologie. Cours de Physiologie.

Ferré, 1882. Étude de la crête auditive. Ann. des sciences nat. Bordeaux.

———, 1885. Contribution a l'étude du nerf auditif. Soc. Zool. de France, x.

Gellé, 1879. Gaz. hebdom de méd. et de chir., 2. s. xvi, 512.

Gottstein, J., 1870. Beiträge zum feineren Bau der Gehörschnecke. Centralbl. f. d. med. Wiss., No. 40, 625–628.

Hardesty, I., 1908. The nature of the tectorial membrane and its probable rôle in the anatomy of hearing. Amer. Journ. Anat., viii, 109–179.

———, 1915. On the porportions, development, and attachment of the tectorial membrane. Amer. Journ. Anat., xviii, 1–73.

Held, H., 1902. Untersuchungen über den feineren Bau des Ohrlabyrinths der Wirbeltiere. I. Zur Kenntnis des Corti'schen Organs u. der übrigen Sinnesapparate des Labyrinths bei Säugetieren. Abhandl. d. k. Sächs. Ges. d. Wiss., Math.-phys. Kl., xxviii, 1–74.

———, 1909. Untersuchungen über den feineren Bau des Ohrlabyrinths der Wirbeltiere. II. Zur Entwicklungsgeschichte des Corti'schen Organs und der Macula acustica bei Säugetieren und Vögeln. Abhandl. d. k. Sächs. Ges. d. Wiss., Math.-phys. Kl., xxxi, No. 5, 195–294.

Henle, J., 1866. Handbuch der systematischen Anatomie des Menschen. Braunschweig.

Hensen, V., 1863. Zur Morphologie der Schnecke des Menschen und der Säugethiere. Ztschr. f. wiss. Zool., xiii, 481–512.

———, 1871. Ueber Boettcher's Entwicklung und Bau des Gehörlabyrinths nach eigenen Untersuchungen. Arch. f. Ohrenh., vi, 1–34.

———, 1873. Besprechungen. Arch. f. Ohrenh., n. F., i, 64; ii, 163.

Huschke, E. Froriep's Notizen, 1832, xxxiii, 33–36. Isis von Oken, 1831, 950; 1833, 675–679; 1834, 107.

Katz, L., 1890. Histologisches über den Schneckenkanal speciell die Stria vascularis. Arch. f. Ohrenh., xxxi, 66–72.

Kishi, J., 1902. Ueber den Verlauf und die periphere Endigung des Nervus Cochleæ. Arch. f. mikr. Anat., lix, 144–179.

Kishi, K., 1907. Corti'sche Membran und Tonempfindungstheorie. Pflüger's. Arch. f. d. ges. Physiol., cxvi, 112–123.

Koelliker, A., 1854. Ueber die letzten Endigungen des Nervus Cochleæ und die Function der Schnecke. Festschr. beim 50-jährigen Doktor Jubiläum Fr. Tiedemann's. Würzburg.

———, 1859. Handbuch der Gewebelehre des Menschen 3. Aufl. Leipzig.

———, 1861. Der Embryonale Schneckenkanal und seine Beziehungen zu den Theilen der fertigen Cochlea. Würzburger naturwissensch. Ztschr., ii, 1–9.

———, 1867. Handbuch der Gewebelehre. 5. Aufl.

Lavdowsky, M., 1876. Untersuchungen über den akustischen Endapparat der Säugethiere. Arch. f. mikr. Anat., xiii, 497–557.

Lebouco, G., 1909. Contribution à l'étude de l'histogenèse de la rétine chez les mammifères. Arch. d'anat. micr., x, 555–605.

Leydig, Franz, 1857. Lehrbuch der Histologie des Menschen und der Thiere. Frankfurt. a. M.

Loewenberg, B., 1868. La lame spirale du limaçon de l'oreille de l'homme et des mammifères. Journ. de l'Anat. et de la Physiol., v, 626–657.

Meyer, P., 1876. Études histologiques sur le labyrinthe membraneux et plus spécialement sur le limaçon chez les reptiles et les oiseaux. Strassburg (Thèse).

Middendorp, H. W., 1867. Het vliezig slakkenhuis in zijne wording en in den ontwikkelden toestand. Groningen.

Nuel, J. P., 1872. Beitrag zur Kenntniss der Säugethierschnecke. Arch. f. mikr. Anat., viii, 200–215.

———, 1878. Recherches microscopiques sur l'anatomie du limaçon des mammifères. Mém. couronnés et mém. des savants étrangers publiés par l'Acad. roy. de Belgique, xlii, No. 1.

Prenant, A., P., Bouin, and L., Maillard, 1911. Traité d' Histologie, ii, 569.

Prentiss, C. W., 1913. On the development of the membrana tectoria with reference to its structure and attachments. Amer. Journ. Anat., xiv, 425–458.

Pritchard, U., 1876. The development of the organ of Corti. Journ. Anat. and Physiol., xiii, 99–103, *and* Quart. Journ. Micr. Sc., n. s., xvi, No. 64, 398–404.

Ranvier, 1889. Traité technique d'histologie. Paris.

Retzius, G., 1872. Anatomische Untersuchungen. I. Stockholm.

———, 1882. Biologische Untersuchungen. No. 6.

———, 1884. Das Gehörorgan der Wirbelthiere. II. Das Gehörorgan der Reptilien, der Vögel und der Säugethiere. Stockholm.

Rickenbacher, O., 1901. Untersuchungen über die embryonale Membrana tectoria der Meerschweinchens. Anat. Hefte, I. Abt., xvi. (Inaug.-Dissert., Basel.)

Rosenberg, E., 1868. Untersuchungen über die Entwickelung des Canalis cochlearis der Säugethiere. Dissert., Dorpat.

Shambaugh, G. E., 1907. A restudy of a minute anatomy of the structures in the cochlea. Amer. Journ. Anat., vii, 245–257.

Spee, F., 1901. Mitteilungen zur Histologie des Corti'schen Organes in der Gehörschnecke des erwachsenen Menschen. Verh. d. anat. Gesellsch. Bonn, 13–23.

Schwalbe, G., 1887. Anatomie der Sinnesorgane. Erlangen.

Tafani, A., 1882. Gli epitelii acustici. Lo sperimentale, I, 587–607.

———, 1884. L'organe de Corti chez les singes. Arch. ital. de Biol., vi, 207–247.

Thing, A., 1917. The formation and structure of the zona pellucida in the eggs of the turtle. Cleveland Med. Journ., xvi, 210.

Van der Stricht, N., 1908. L'histogenèse des parties constituantes du neuro-épithélium acoustique, des taches et des crêtes acoustiques et de l'organe de Corti. Arch. de Biol., xxiii, 541–693.

Van der Stricht, O., 1909. Le neuro-épithélium olfactif et sa membrane limitante interne. Mém. couronnés et autres mém. publiés par l'Acad. roy. de Méd. de Belgique, xx, fasc. 2.

Vasticar, E., 1909. Notes d'histologie. Étude sur la tectoria. Journ. de l'Anat. et de la Physiol., xlv, 459, 473.

———, 1911. Notes d'Histologie. Les sangles des cellules de soutènement de l'organe de Corti. Journ. de l'Anat. et de la Physiol., xlvii, 60–99.

Vernieuwe, 1905. Contribution à l'étude du développement embryonnaire et post-embryonnaire du limaçon des mammifères et de l'homme. La Presse oto-laryngol. belge, iv, 241, 289.

Waldeyer, W., 1872. Hörnerv. und Schnecke. *In* S. Stricker, Handbuch der Lehre von den Geweben der Menschen und der Thiere, Leipsig, ii, 915–963.

von Winiwarter, A., 1870. Untersuchungen über die Gehörschnecke der Säugethiere. Sitzungsb. d. k. Akad. d. Wiss., Wien, Math.-naturw. Cl., lxi, 1. Abt., 683–714.

EXPLANATION OF FIGURES.

[All figures are photo micrographs taken at a magnification of 750 diameters, except figure 3, which is 400 diameters, and figure 25′, which is 800 diameters. By the reproduction they have been reduced one-tenth.]

ABBREVIATIONS.

ar.,	areolar tissue.
bm.,	basement membrane.
cp.,	central corpuscle.
cy.,	cylinders or prisms of membrana tectoria cut lengthwise.
cy′.,	cylinders or prisms of membrana tectoria cut transversely.
cy″.,	cylinders or prisms cut tangentially.
d′., d″., d‴., d^{iv}.,	apices of the four rows of Deiters cells.
ep.,	epithelial cells.
essp.,	epithelial cells of the future sulcus spiralis.
f.,	clear cytoplasmic fields.
f′.,	dark cytoplasmic fields.
f″.,	filamentous fields.
fb.,	fibrillar bundles or superficial segments of the bodies of outer pillars.
gr.,	greater epithelial ridge.
hs.,	superficial horseshoe-like elements, the bases of the acoustic hairs.
ic.,	intraepithelial connective-tissue.
ih.,	apices of the inner hair-cells.
ip.,	apices of the inner pillars.
is.,	apices of the inner supporting cells.
l.,	longitudinal lines of the recently formed parts of membrana tectoria.
l′.,	transverse lines of the recently formed parts of membrana tectoria.
lr.,	lesser epithelial ridge.
m.,	superficial mosaic.
mcr.,	superficial mosaic of crista spiralis.
mg.,	superficial mosaic of greater epithelial ridge.
mh.,	superficial mosaic of Hensen cells.
mi.,	mitotic figures.
min.,	superficial mosaic of axial segment of the greater ridge.
ml.,	superficial mosaic of lesser ridge.
ms.,	striated membrane or apices of inner pillars.
mt.,	membrana tectoria.
mt′.,	recently formed part of membrana tectoria.
mter.,	membrana tectoria formed on the surface of the crista spiralis.
mtg.,	membrana tectoria formed on the surface of the greater ridge.
mtl.,	membrana tectoria formed on the surface of the lesser ridge.
n.,	nuclei.
nb.,	nuclear bands of epithelial cells.
nd′., nd″., nd‴.,	nuclei of the three rows of cells of Deiters.
ner.,	nerve-fibers.
ni.,	nuclei of the inner hair-cells.
nip.,	nuclei of the inner pillars.
nisu.,	nuclei of the inner supporting cells.
noh′., noh″., noh‴.,	nuclei of the three rows of outer hair-cells.
nop.,	nuclei of the outer pillars.
ns.,	nuclei of the sensorial cells.
nsu.,	nuclei of the supporting cells.
oh.,	outer hair-cells.
oh′., oh″., oh‴., oh^{iv}.,	apices of the four rows of outer hair-cells.
op.,	apices of the outer pillars.
pb.,	protoplasmic bands of epithelial cells.
per.,	periosteal membrane.
pr.,	prolongations of connective-tissue cells.
s′., s″., s‴.,	three different successive planes of recently formed part of the membrana tectoria at surface of the lesser epithelial ridge.
sf.,	sensorial fields.
sph.,	attraction sphere.
ssp.,	future sulcus spiralis.
st.,	subepithelial part of teeth of Huschke.
suf.,	fields of the supporting cells.
t.,	teeth of Huschke.
tb.,	terminal bars.
tb′.,	dense part of the membrana tectoria.
th″.,	terminal bars alternately thicker and thinner.
zd.,	zona dentata of the crista spiralis.
zp.,	zona papillaris of the crista spiralis.

DESCRIPTION OF PLATES.

Plate 1.

1. Photograph of transverse section through tympanic wall of cochlea near apex. New-born dog. Fixation, trichloracetic acid; stain, iron hematoxylin, Congo red.
2. Photograph of transverse section through second turn of spiral duct. Pig embryo 93.5 mm. Trichloracetic acid; iron hematoxylin, Congo red.
3. Photograph of transverse, slightly oblique section through the greater and lesser ridges of second turn of cochlea, New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
4. Photograph of section tangential to surface of the two epithelial ridges in second turn of cochlea. New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
5. Photograph of section tangential to surface of the two epithelial ridges between second and third turn of cochlea. New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
6. Photograph of section tangential to surface of crista spiralis and the greater epithelial ridge. Pig embryo 137 mm. Bouin's fluid; iron hematoxylin, Congo red.
7, 7′. Photographs of a section tangential to the surface of the organ of Corti in the adult bat (*Vespertilio fuscus*) Bouin's fluid; iron hematoxylin, Congo red.
8. Photograph of section tangential to surface of crista acustica. New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
9. Photograph of section tangential to surface of crista acustica in adult bat (*Vespertilio fuscus*). Zenker's fluid; iron hematoxylin, Congo red.
10. Photograph of section tangential to the surface of crista acustica in adult white rat. Trichloracetic acid; iron hematoxylin, Congo red.

Plate 2.

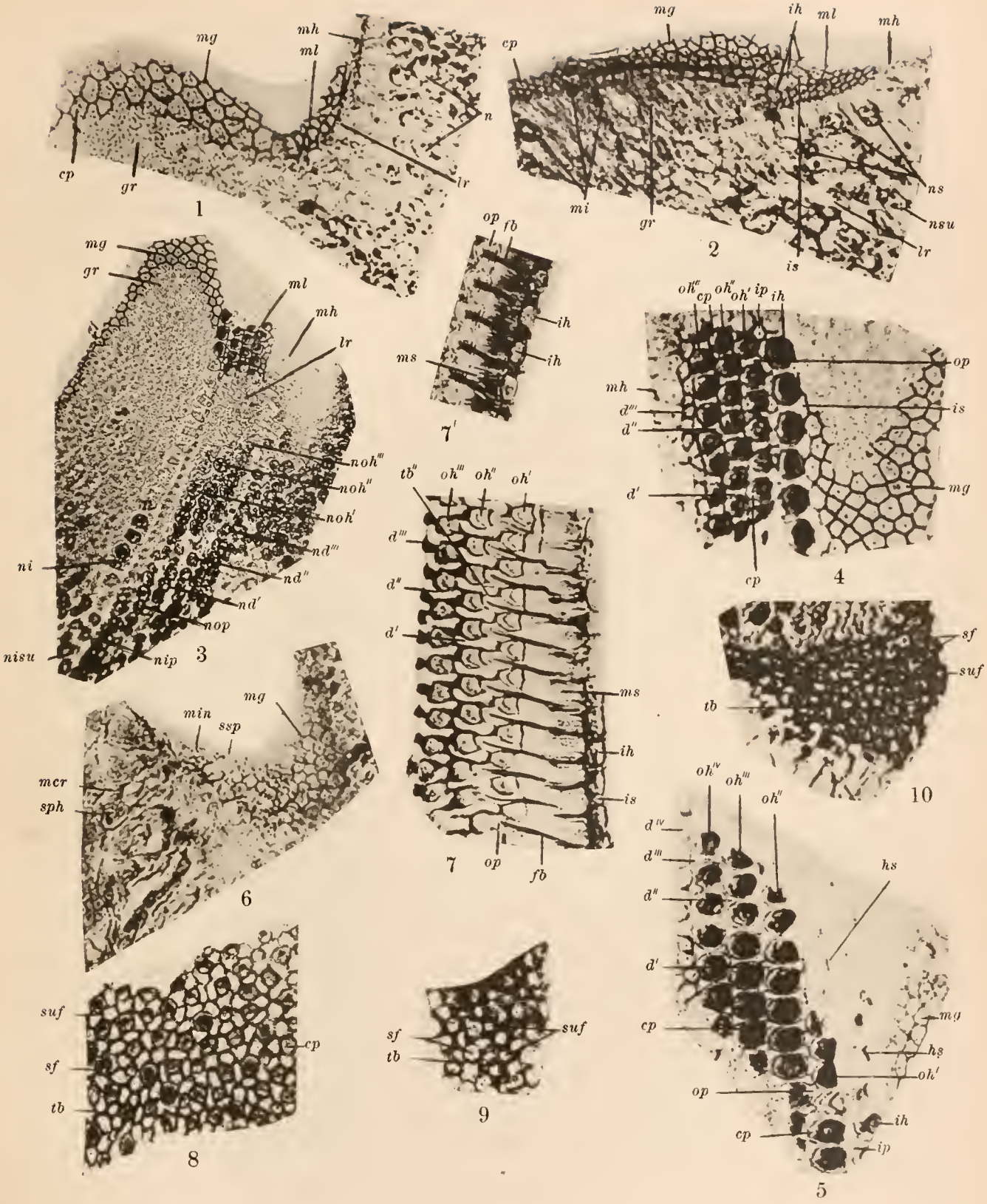
11. Photograph of section tangential to surface of macula acustica. Adult mouse. Trichloracetic acid; iron hematoxylin, Congo red.
12. Photograph of section vertical to surface of the crista spiralis of second turn of cochlea. Pig embryo 127 mm. Bouin's fluid; Mallory's stain.
13. Photograph of section tangential to the surface of the crista spiralis of the third turn. Pig embryo 95 mm. Zenker's fluid; iron hematoxylin, Congo red, light green.
14. Photograph of section tangential and slightly oblique to surface of crista spiralis. Pig embryo 127 mm. Trichloracetic acid; Mallory's stain.
15. Photograph of section tangential to surface of crista spiralis. Pig embryo 137 mm. Bouin's fluid; Mallory's stain.
16. Photograph of section vertical to surface of crista spiralis. Pig embryo 127 mm. Trichloracetic acid; Mallory's stain.
17, 18. Photographs of section tangential to surface of crista spiralis of the third turn. New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
19. Photograph of section tangential to the crista spiralis. Pig embryo 127 mm. Trichloracetic acid; iron hematoxylin, Congo red.

Plate 3.

20. Photograph of section vertical to surface of crista spiralis. Pig embryo 190 mm. Bouin's fluid; Mallory's stain.
21. Photograph of section vertical to surface of crista spiralis. Young dog, age 4 months. Trichloracetic acid: iron hematoxylin, Congo red, light green.
22. Photograph of section tangential to surface of crista spiralis. Pig embryo 190 mm. Trichloracetic acid; iron hematoxylin, Congo red.
23. Photograph of section tangential to surface of crista spiralis in adult bat (*Vespertilio fuscus*). Zenker's fluid, iron hematoxylin, Congo red.
24. Photograph of section tangential to surface of crista spiralis. Young dog. Bouin's fluid; iron hematoxylin; Congo red.
25, 25′, 26, 26′. Photographs of sections tangential to surface of greater ridge. New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
27. Photograph of section tangential to surface of greater ridge. Pig embryo 93.5 mm. Fixation by uranium nitrate method of Ramon y Cajal.

Plate 4.

28. Photograph of section tangential to surface of the two epithelial ridges and the crista spiralis and their membrana tectoria, between the second and third turns of cochlear duct. New-born dog. Trichloracetic acid; iron hematoxylin, Congo red.
29, 30. Photographs of sections vertical to surface of the greater epithelial ridge. Pig embryo 95 mm. Zenker's fluid; iron hematoxylin, Congo red, light green.
31. Photograph of section vertical to the surface of the greater epithelial ridge. Pig embryo 95 mm. Zenker's fluid; Mallory's stain.
32. Photograph of section tangential to surface of the greater epithelial ridge. Pig embryo 127 mm. Trichloracetic acid; iron hematoxylin, Congo red.
33. Photograph of section tangential to surface of organ of Corti and its superficial membrana tectoria. Pig embryo 150 mm. Zenker's fluid; iron hematoxylin, Congo red, light green.
34. Photograph of section tangential to surface of crista spiralis. Pig embryo 127 mm. Trichloracetic acid; iron hematoxylin, Congo red.
35. Photograph of section tangential to surface of membrana tectoria. Adult rat (*Mus decumanus*). Bouin's fluid; iron hematoxylin, Congo red, light green.
36. Photograph of section tangential to surface of membrana tectoria. Pig embryo 150 mm. Bouin's fluid; Mallory's stain.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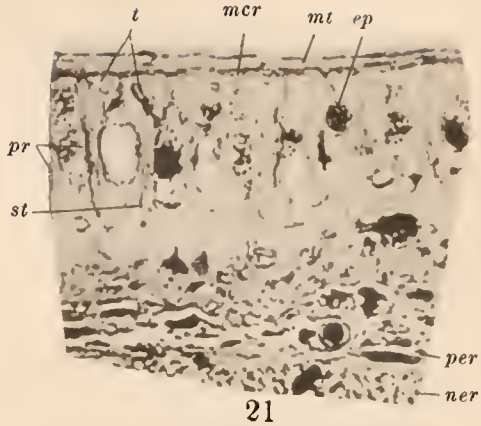

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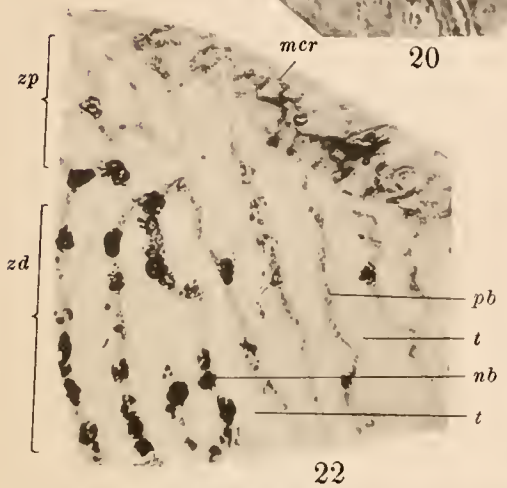

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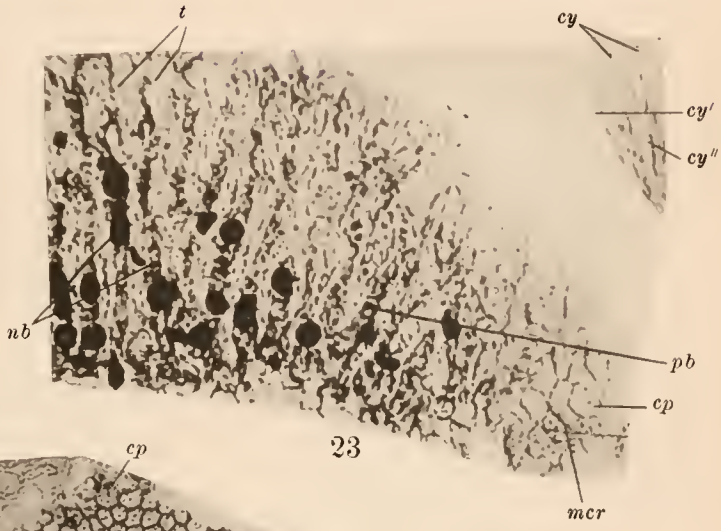

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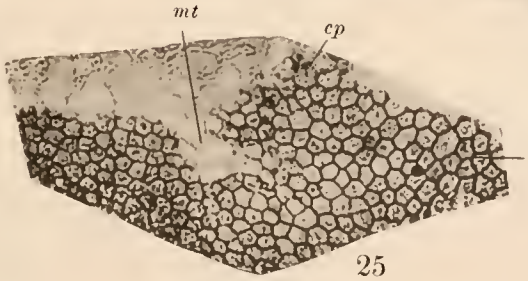

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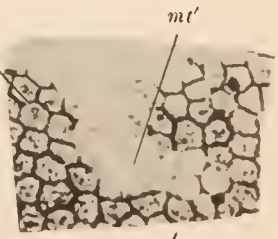

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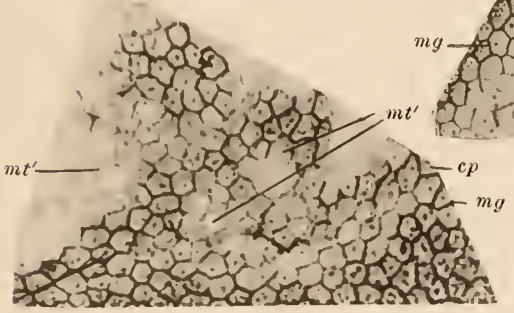

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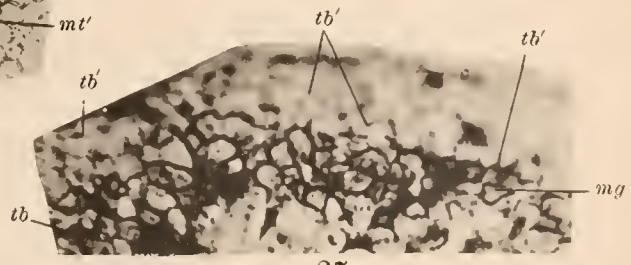

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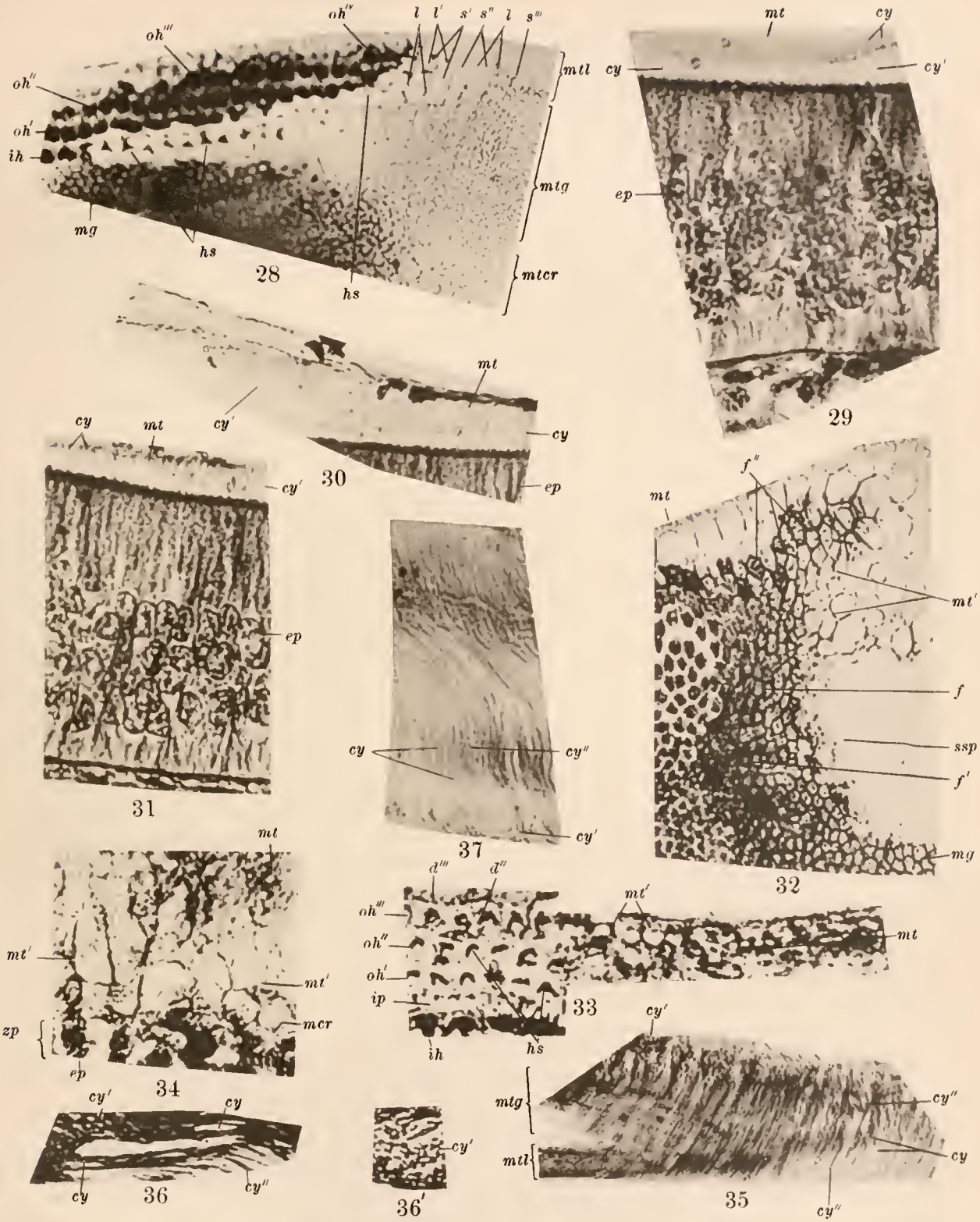
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CONTRIBUTIONS TO EMBRYOLOGY, No. 22

STUDY OF A HUMAN SPINA BIFIDA MONSTER WITH ENCEPHALOCELES AND OTHER ABNORMALITIES.

By Theodora Wheeler.

With four plates.

STUDY OF HUMAN SPINA BIFIDA MONSTER WITH ENCEPHALOCELES AND OTHER ABNORMALITIES.

By Theodora Wheeler.

The specimen described in this study is a human female monster with spina bifida, in which there is total subcutaneous involvement of the spine and a defective occiput. The thoracic and cervical regions of the spine are much shortened, and encephaloceles and numerous other abnormalities are present. The type, though a rather unusual variety of spina bifida, occurs frequently enough to have been recognized and grouped by itself for some time past, and to this group the term *iniencephaly* has been applied. Because of the striking appearance of these specimens, one or more are usually to be found in any museum of pathology. In the embryological collection of over 1,600 specimens belonging to the Department of Embryology of the Carnegie Institution of Washington, the only example is the one presented in this paper, No. 862*a*. It was through the courtesy of the Bridgeport General Hospital that this specimen was obtained.

Only a short review of the literature on spina bifida will be given here. More complete historical accounts with extensive bibliographies are to be found in articles by Kermauner and by Ernst in Schwalbe's Morphologie der Missbildung (1909), and also in a chapter on spina bifida by Tillmann, in his volume of Deutsche Chirurgie, v. 62*a* (1905). The earliest references to many teratological conditions are supposed to be found in folklore and in mythological tales of centaurs, cyclops, mermaids, and such creatures, and it has been suggested that among such stories may likewise be found the first record of spina bifida. Possibly the hairy and cloven-hoofed satyr was originally a fairly normal individual with spina bifida, hypertrichosis, and club feet, whose abnormalities gradually developed through excited hearsay into the hind-quarters of a beast. Even in recent times, in connection with scientific work on this condition, such inaccuracies have only too frequently been paralleled by superficial observations and indefinite speculations. However, it is not surprising that a good deal of vagueness has existed in regard to spina bifida, as the subject-matter includes widely dissimilar and very complicated conditions.

As described among human forms, two chief types are distinguished: the flat-spine type (rachischisis, spina bifida aperta) and the subcutaneous type (cystic, occulta). In both of these forms the greatest variations exist as to location and amount of spinal involvement. In some instances only a single segment is affected; in others the whole spinal column, together with the cranium, may be involved. Combinations of these two forms are to be found, and also conditions where the two varieties merge into one another. Associated with every type of the condition are found innumerable other abnormalities.

Owing to this mass of complicated material and to the widely different nomenclatures used by the large number of investigators who have worked on the problem, the literature is enormous and rather confused. The classification is still very superficial. In teratology, as in general pathology, the trend has been to supplant classifications based on regional distribution by those having an etiological basis. There exists still in the literature on spina bifida a great deal of the former method. This is due to the fact that until quite recently study has been of external form alone, from which method only a crude regional classification can result. By the application of the more penetrating methods of modern anatomy, embryology, and experimental biology, progress has been made toward etiological classification.

In 1881 Koch assembled a number of different forms of spina bifida. He pointed out the distinction between the flat-spine form, in which the spinal cord is uncovered (spina bifida aperta), and the cystic or subcutaneous form, in which the soft parts have joined but the bony arches remain ununited. He attributed a later formative period to the subcutaneous than to the open form. In 1886 von Recklinhausen presented over 30 specimens of spina bifida and focussed attention especially upon the pathological anatomy of the central nervous system and its membranes in the fetal and older forms. By thoroughly analyzing the conditions met with and applying the conception of arrested development, he was able to offer reasonable interpretations for much of the developmental mechanism which up to that time had not been understood. Contemporaneously with these two writers, and since their time, many aspects of the subject have been studied. The surgical treatment of spina bifida has been taken up by many, notably Bayer, Hildebrand, and Muscatello. Other authors have described special types of the abnormality Among these may be mentioned Lewis's paper on iniencephaly. He collected 23 cases similar to the one herein described, which show some of the variations presented by this special form. In the literature are to be found fairly numerous descriptions of young specimens with spina bifida. In "A study of the causes underlying the origin of human monsters" (1908), Mall describes 12 from his collection and cites several others from the literature. An interesting 8 mm. ferret embryo with localized cervical hydromyelia was described by Good in 1912.

Experimental studies on the lower animals have formed a very important source of information with regard to the open variety of spina bifida. In Mall's paper a review of the literature on the subject up to 1908 is given. The work of Hertwig and Morgan has attracted especial attention, the former showing that external agents causing delay in the closure of the blastopore can bring about embryological spina bifida. The work of the latter author has been interpreted as pointing toward NaCl as the definite etiological agent, as he was able to produce a delayed closure of the blastopore in frogs' eggs through the use of a 0.6 per cent solution of NaCl. Embryological spina bifida has also been produced occasionally in chicks by overheating and various other methods. Working with frogs' eggs, by ultra-violet-ray exposures Baldwin (1915) obtained a condition of doubled and closed neural canal and sometimes doubled cord. His specimens were usually

two-tailed. He referred to them as spina bifida and gave a clear explanation of the mechanics of the process producing them. However, the relation of this type of spina bifida to the more ordinary condition of a single open neural canal is not altogether plain, and his suggestion that "imperfect oxidation" causes spina bifida does not further clarify the question.

The earliest picture of the subcutaneous type of spina bifida with which we are familiar is that encountered in embryos around the 10 mm. stage of development, in which the neural tube is everywhere closed, showing, however, a greater or less area of enlargement. Such a state has not as yet been experimentally produced. Several explanations have been advanced to account for it, none of which are satisfying, nor substantiated by evidence. One suggestion is that the enlargement of the neural tube is due to the fact that dorsally it remains attached to the ectoderm (non-separation of the membrana reuniens). On the other hand, it has been suggested that the neural tube becomes enlarged because of increased pressure from the contained fluid. In this connection it would seem that when the affected areas are limited in extent they are in some way connected with the curvatures of the body, since such areas usually occur in the neck or sacral region, where, in the embryo, the curves are most pronounced. The process is supposed to be one of subsequent pressure of the hydromyelia on the surrounding parts, thus inhibiting the development of cartilage and bone. With our advance in knowledge regarding the circulation of the cerebro-spinal fluid, some of the most puzzling features presented by subcutaneous spina bifida will probably be satisfactorily explained. The work of Weed on the normal cerebro-spinal fluid circulation is most helpful, supplying as it does for the first time an adequately correlated picture of the formation and extension of the cerebro-spinal fluid with the differentiation of the perimedullary mesenchyme to form the meninges. In the meantime, any discussion concerning the etiology of subcutaneous spina bifida is entirely theoretical. Suggestions have been made that it may arise directly from the open spina bifida form; again, that it may be the result of some entirely different process, or that both forms may be produced by the same pathological agent acting at a different stage of development. While it has been generally assumed that the open and subcutaneous forms of spina bifida are related, this has never been proved. A more definite picture of each process must be obtained before we can know the nature of the relation, or whether there is such a relation, existing between the two. The literature on the subject gives the impression that, although sound facts and more or less sound theories regarding spina bifida have multiplied, there is much that is not clear and that must be understood before we can have a comprehensive insight into the processes producing it. That this information may be gained through a closer embryological study seems probable.

The study of the specimen dealt with in this paper has been made chiefly along morphological lines. Only a meager clinical history regarding it was obtainable; the child was illegitimate, was born spontaneously at full term, and lived only a few hours. Its external form is shown in figures 1, 2, and 3, and various measurements are given in table 2. Externally, the most marked abnormality is the

extreme dorsal flexion and shortening of the trunk. The head is drawn back close to the sacral region. The chest and abdomen are unusually prominent. The arms and legs are symmetrical and well developed, but the shoulders are hunched up and lie far forward, close to the cheeks. The face is directed upward, which throws the top of the head back so that the vertex lies level with the raised shoulders. The neck is obliterated and the chin and chest lie in one plane. The features are well formed with the exception of lack of prominence of the chin and deformity of the ears. Figures 1 and 8 show the right ear. The deformity of the left ear is similar. The anthelix is pushed outward so as to be unusually prominent; the tragus is shifted medially and upward, so that it lies opposite the concha; the antitragus lies below it, pressed against the cheek. Darwin's tubercle is present. The external auditory meatus is patent and the parts of the middle and inner ear prove on dissection to be well developed. The whole external ear is considerably narrowed, as is indicated by as low a physiognomical index as 48.5. The average physiognomical index of the right ear of 14 white infants under 3 weeks of age, in the obstetrical ward of the Johns Hopkins Hospital, was found to be 69.1, varying between 62.5 and 78.7. Measurements made by Dr. A. H. Schultz of 4 dead white infants not older than 1 month showed the physiognomical index of the right ear to be 65.0, with a variation between 60.0 and 73.1. Though the physiognomical index shows a rather wide variation due to the great flexibility of the ear cartilage in infants, nowhere in the small group of available normal cases is it nearly so low as in his specimen. The ear deformity is apparently caused by pressure upon and twisting of the external parts of the ear during their development by the backward-bent head and the shoulders which lie close on either side. Marx describes a deformed ear which he designates as "Wildermüthsche Ohr," in which the anthelix is very prominent. From the base of each ear a crease in the skin extends for 3 cm. medially under the chin to within 2 cm. of the midline, as is seen in figure 2.

INTEGUMENT.

The dome of the head is narrowed and flattened, and is covered with light-brown hair, 1 cm. in length. Just above and behind the ears the hair is 2 cm. long and is quite thick. Across the middle of the forehead at the hair margin is a narrow raised ridge of puckered skin, 2 cm. long. A section through this area shows the structures of the skin to be well developed and similar to the adjoining normal skin, except that where the surface is raised the papillæ are somewhat flattened underneath. The ridge is probably the result of rough handling before fixation.

From the back of the head protrude three encephaloceles in a horizontal line, extending from a point 6 cm. behind the tip of the left ear to a point 2 cm. behind the tip of the right ear. These are best displayed in figures 1 and 3. They measure 13 cm. horizontally along their superior margin, and the vertical diameter is 4.3 cm. The midline of the specimen comes between the left and middle encephaloceles. In the midline their vertical diameter extends from a point 3.6 cm. below the vertex to within 6 cm. of the anus. The middle swelling is the largest of the

three and is cone-shaped, whereas the other two are smaller and hemispherical. All are of soft consistency. The large conelike swelling measures 6 cm. from the superior margin to its tip and 2.5 cm. from tip to inferior margin, its base being circular and measuring 4.3 cm. in diameter. This cone lies pendant over the back; the proximal half of its superior surface is covered with scalp and fine brown hair 2.5 cm. long. A strip of coarser hair of the same length follows the median margin of the swelling to its lowest point. The covering of the distal half of the superior surface and the entire inferior surface resembles smooth, fine-grained leather. No hairs are present, but it is dotted with minute pores which on microscopical examination are seen to be the mouths of sweat-ducts. The wall of the sac is 3 mm. thick, a section through which shows an extremely thin layer of epidermis lying immediately over a vascular connective tissue, containing the sweat-glands mentioned above, but no hair follicles. There are two oval nævi near the tip of the sac, which in the gross resemble scars. These lie in the same long axis directed laterally through the tip of the sac. The smaller of the two is 5 mm. to the left of the tip and measures 7 by 3 mm. The larger lies 10 mm. to the right of the tip and measures 25 by 17 mm. The color of these areas is lighter than the surrounding tissue and the surfaces are stiff, smooth, and slightly raised. In their neighborhood the thickness of the sac wall is increased to 9 mm. Histologically the epithelium is lacking here and a very vascular connective tissue forms the raised surface. The lining of the upper part of the sac is smooth fibrous tissue continuous with the dura of the main cerebro-spinal cavity. Near the tip, however, it is made up of shaggy strands of blood-vessels whose complicated, interweaving pattern is like the early capillary plexus of the dura, as described by Streeter (1915). This suggests that the irregular vascularization in this region may be due to arrested development of the vascular system. There is marked engorgement of these vessels and congestion in all the tissues. Part of this extreme engorgement was probably caused by birth trauma.

The left encephalocele measures 5 by 4 cm. and protrudes 1 cm. from the surface. Its upper half is covered with fine hairs and the lower half with normal-appearing skin. The wall is 1 to 2 cm. thick, composed chiefly of a layer of subcutaneous fat. On its left lower border there is a rounded bleb of porous, wrinkled skin 1 cm. in diameter, over which are scattered a few hairs 2 cm. long, and which contains around its depressed circumference a much thicker growth of similar hairs. A section through the wrinkled skin shows that it lies over a funnel-shaped canal, the wider mouth of which extends down through 1.5 cm. of subcutaneous fat to the subdural space, where the canal becomes narrow. This canal is filled with fluid and contains a few blood-vessels supported by loose connective-tissue septa. Its walls are formed of rather dense connective tissue.

The right encephalocele is 4 cm. in diameter at its base, and the surface, which is covered with hairy scalp, is but slightly raised above the adjoining structures. Its wall, consisting of epidermis, connective tissue, and fat, is but 3 mm. thick.

A summing up of the integument findings shows that both normal skin and scalp are found over the areas adjoining the encephaloceles and over parts of the

encephaloceles as well. In addition to this, in the adjoining areas there are hypertrichosis and thickened subcutaneous fat, varying from 5 to 25 mm. in thickness. The wall of the large encephalocele varies from 3 to 9 mm. in thickness and is formed by angiomatous tissue covered with a thin layer of epidermis penetrated by sweat-glands. In two places near the tip of the sac nævi are formed by the vascular tissue extending to the surface. The walls of the small sacs vary from 3 to 20 mm. and are formed chiefly by subcutaneous fat covered with scalp. On the left a pore and canal pierce through to the subdural space.

Spietseka in 1894 collected the various forms of skin changes associated with spina bifida. Besides the varieties here found, he described pigment blotches and such a marked increase of fatty tissue as to amount to lipomata. In an article on skin anomalies by Bettmann, in Schwalbe's Morphologie der Missbildungen, nævi are noted as among the most frequent anomalies.

METHODS.

A sagittal section was made of the specimen under discussion (see fig. 9). The spinal column shows extreme lordosis, undeveloped arches throughout, and shortening and fusion of the upper vertebræ. The central nervous system is very much disturbed, a large part of it having slipped down below the cranium, through a much enlarged foramen magnum. This portion lies on the thoracic and lumbar vertebræ and protrudes into the sacs already described. The brain and cord were removed, and a clay impression was made of the entire space occupied by the central nervous system. This was then cast in wax and photographed, as shown in figures 4, 5, 6, and 7. By the help of this model the general shape taken by the central nervous system was demonstrated and the study of its internal arrangement and relation to other structures was facilitated. The consideration of these will be taken up later in this paper.

In the sagittal section, thick subcutaneous pads of fat are seen in the undifferentiated region of the neck between chin and thorax, above the symphysis, and over the sacral region. Dissection shows this subcutaneous fat to be likewise particularly abundant over the back and shoulders. There is also found an extreme grade of undeveloped or split soft palate, associated with which is a bilateral anlage of the uvula, that on the left side being shown in figure 9*a*. Consideration of the normal development of the soft palate will help to indicate how this defect originated. It is generally agreed that at a very early date the tongue occupies the area which is later occupied by the septum and palate. The normal rearrangement of these parts to their final positions is accomplished by medial growth of the palate and downward growth of the septum, associated with independent shifting of the tongue. If for any reason the tongue can not withdraw, the palate remains split to a greater or less degree. That such a cause was operative in this specimen seems likely; the distorted position of the cervical spine might easily have caused a crowding in the adjoining pharyngeal region and so prevented the tongue from receding.

SKELETON.

A dissection of the skeleton was made, the vertebræ and ribs being left connected by their ligaments, so that the specimen could be easily mounted. To facilitate handling, two transverse cuts in the skeleton were made at the level of the first thoracic and first lumbar vertebræ. A study of the skeleton shows marked maldevelopment and distortion, as may be seen in figures 10, 11, and 12. The axial skeleton is most affected, the arches of all the vertebræ being defective; these are open posteriorly in the midline and are flattened outward, forming wide anterior support for the central nervous system. In the cervical and thoracic regions the bodies of the vertebræ are fused, shortened, and dorsally flexed, so that the spine is bent almost double. The occiput actually rests on the gaping vertebral arches and fuses with them.

Viewing the occiput in figures 13 and 14, the inferior and medial two-thirds of the squama occipitalis is seen to be defective. A bilateral bony excrescence on its dorsal surface, near the defective medial margin of the squama and close to its junction with the partes laterales joins it to the everted arches of the second lumbar vertebra on the left side and to the first lumbar vertebra on the right. The defect of the squama in the midline, together with a widening of the angles formed by the junction of the pars basalis with the partes laterales, has greatly increased the size of the foramen magnum. This is oval in shape and measures 4.5 by 3.7 cm. The long diameter is antero-posterior, and posteriorly it slants slightly to the left. For purposes of comparison the size of a normal foramen is indicated in figure 13 by means of dotted lines. The large foramen resembles that of the chondrocranium at a very early stage of development. The participation of both the squama occipitalis and the vertebral arches in the midline defect, as it exists here, has been regarded as teratological evidence of the homology of these parts, and probably has been a factor in advancing the opinion, which has slowly gained ground, that some cranial defects, even when existing alone, belong in the same category with certain vertebral abnormalities.

The two partes laterales are well formed and but slightly asymmetrical. The left jugular process is more marked than the right. On the left inferior surface directly under the jugular process there is a cartilaginous prominence which meets the tip of the transverse process of the underlying atlas. The hypoglossal foramen on the left side is a single canal, and while the right hypoglossal foramen has a single perforation on the medial surface of the pars lateralis, it has a double exit on the outer surface of the bone. A small rod of bone divides it into a smaller anterior and a larger posterior foramen, as is demonstrated in figure 11. A division similar to this has been observed frequently in embryological studies and appears on the left side in a skull of a human fetus modeled by Macklin. The condition is of rather frequent occurrence. Lillie gives a ratio of 14 per cent complete division and 36 per cent indicated division, out of 305 left and right canals examined by him. The explanation generally offered is that it is persisting tissue from primitive cranial divisions which usually disappear at a very early stage.

In our specimen the pars basalis of the occiput is oval and asymmetrical along its inferior margin, as shown in figure 11, and measures 18 by 14 mm. Its sphenoidal margin is 6 mm. thick, and the thickness of the bone elsewhere is 3 mm. Its posterior surface is slightly concave, there being a rather deeper depression immediately under the sphenoidal articulation than elsewhere. The anterior surface of the pars basalis is nearly flat. The inferior margin has a notch near the midline and on either side of this the bone projects downward, 2 mm. on the right and 4 mm. on the left. A slit-like foramen 2.7 mm. wide directed forward and upward pierces the pars basalis near its center. On each temporal bone the eminentia arcuata is very prominent and the fossa subarcuata deeply depressed below. The ear ossicles are well developed and the other relations of the bone are normal. With the exception of the small size of the cranial vault the rest of the skull is well formed.

The bodies of all the cervical and thoracic vertebræ and the dorsal surface of the first lumbar vertebra are fused together in a bent and irregular central plate of cartilage containing irregular ossification centers. The roots of the arches and the ribs project from this plate. The relations of the various parts are shown in figures 10, 11, and 15. At the superior end of this plate the foveal surfaces of the atlas and its transverse processes are distinguishable, but, as may be seen in figure 10, both posterior and anterior arches are lost. The foveæ are shifted to the right in relation to their transverse processes, as may be seen in figure 11. This shifting causes the right atlantal transverse process to lie immediately under the fovea. The left is uncovered by the fovea on that side, but is fused at its tip with the left pars lateralis.

Viewed from the side in figure 12, the cervical and lower thoracic portions of the central vertebral plate form the two arms of a wide-mouthed U, while the bent base of the U occurs in the plate from the level of the first to the sixth ribs. Besides this marked lordosis, there is a very slight lateral bend which shows in the dorsal view of the skeleton (fig. 10), giving the vertebral plate a slightly curved S-shape. (This condition of scolisosis and lordosis in varying degrees is very frequently noted in the extreme forms of spina bifida.) The concavity at the right margin of the vertebral plate is opposite the first rib and at the left opposite the sixth rib. From the central plate of this specimen throughout its extent the radices project outward on both sides and formed between them are two uneven rows of intervertebral foramina. The processes are tiny spicules of bone in the cervical and upper thoracic region, becoming larger in the lower part of the column. In the cervical region 7 radices are distinct and 12 in the thoracic region. The former could not all be shown in the drawings.

The arches of both cervical and thoracic regions are everted and fused. This formation, together with the antero-posterior bend of the plate, makes a rather deep pocket of bone which contains parts of the much disturbed central nervous system. The lumbar and sacral vertebral column is much less affected than the upper part. The dorsal part of the first lumbar vertebral body is fused with the thoracic vertebræ, its ventral surface, however, being distinct. The four lower

lumbar and the five sacral vertebral bodies are well formed, as are the transverse processes of all the lumbar vertebræ and the partes laterales of the sacrum (figs. 10 and 12). The first four lumbar arches are everted, as are the thoracic arches, though individually they are distinct and not fused. The fifth lumbar and the five sacral arches are incomplete, but project medially toward one another and are not everted. The lumbar column is 4.3 cm. long and the sacrum 3 cm. in length. The coccyx is composed of four segments, which measure 1.6 cm. and are bent to the left. In studying the proportions of the vertebral column, Aeby's tables of relations in normal vertebral columns in the new-born were used, with the results shown in table 1.

TABLE 1.—*Comparison in millimeters of the vertebral lengths of specimen with those given by Aeby for normal new-born.*

	Total.	Cervical.	Thoracic.	Lumbar.
Aeby (normal)....	176.5	45.1	83.9	47.5
No. 862*a*.....	105.0	15.0	45.0	43.0

A comparison of these vertebral lengths shows the lumbar portion of this specimen to be within the limits of normal, though near the minimal margin. The cervical portion is less than half, and the thoracic portion a trifle more than half the length of the normal. Aeby gives 26.4 mm. for the transverse diameter of the atlas, 12.2 mm. for the width of the body of the sixth thoracic vertebra, and 17.5 mm. for that of the fifth lumbar. In this specimen the lateral limits of the foveæ are 31.0 mm. and the width of the transverse processes of the atlas 38.0 mm. The width of the vertebral plate in the midthoracic region is 23 mm. and the width of the fifth lumbar vertebra is 21 mm. These differences show an irregular widening process to have taken place in the vertebral bodies themselves, the change being most marked in the thoracic and cervical regions. The absence of lateral pressure from ununited arches must have been an important factor in this broadening process.

There are twelve ribs on each side which have undergone considerable disturbance. On the right, the first six are fused near their bases (figs. 10, 11, and 15). The second rib terminates at the end of its proximal third in a plate of bone by which it is joined to the first and third ribs. On the left, the fifth to ninth ribs are crowded together in their proximal half (figs. 10, 12, and 15). The fifth and sixth have but one costal cartilage between them. The sixth and seventh ribs are fused for a few millimeters just proximal to their termination. Further fusion occurs in pairs at the bases of the following ribs: on the right, between seventh and eighth, ninth and tenth; on the left, between the first and second, third and fourth. This shows on the ventral surface in figure 15.

The sternum, as seen in figure 16, has four ossification centers near the median line at the level of the first costal cartilage and of the first, second, and third left intercostal spaces. There are six costal cartilage connections on each side. The last on each side, however, belongs to the seventh rib. The discrepancy occurs on the right side through the aborted second rib and on the left side through the

fifth and sixth, having but one cartilage between them. The first and seventh costal cartilages of the two sides are opposite each other. The arrangement of the other cartilages is such that the third to the fifth on the left side are from 0.5 to 1 cm. lower than the corresponding cartilages on the right, yet not quite opposite the succeeding one. A small cartilaginous knob (2 by 5 by 3 mm.) above the manubrium is a persistent episternum. The measurements of the sternum are given in table 2.

TABLE 2.—*Dimensions in centimeters.*

	cm.
Body lengths:	
Vertex–anus	14.0
umbilicus	32.0
Lower hair border–anus (length of back)	10.0
Head:	
Circumference of head	32.5
Biparietal diameter	8.5
Anterior fontanel	2.7 cm. transverse by 2.5
Posterior fontanel	1.6 cm. " by 1.7
Face, vertical length (border of hair to chin)	9.5
Clear breadth (from free edges of tragi)	9.2
Eyes apart	1.8
Nose across	2.3
Mouth across	2.0
Trunk:	
Circumference at umbilicus, passing around the back at base of middle sac	33.0
Distance across shoulders	12.0
Nipples apart	6.4
Trunk—*Continued.*	cm.
Diameter of right nipple	0.9
left nipple	.7
Sternum, episternum, and zyphoid:	
Length of sternum with episternum and zyphoid sternum alone	6.9 6.0
Width of sternum	1.0
Thickness of sternum	.3
Length of episternum	.3
Length of zyphoid	9.6
Extremities:	
Upper arm (circumference of both left and right)	9.0
Lower arm (circumference of both left and right)	7.0
Hand with middle finger	6.0
Right trochanter—heel	20.9
Right foot	7.5

The two scapulæ which are shown in figures 18 and 20 are distorted, as will be seen by comparison with figures 17 and 19, representing normal left and right scapulæ. In both the pathological bones the supraspinous portions are poorly formed and the inferior vertebral margins are concave. Graves designates a concavity of the vetebral margin of the scapula as scapula scaphoidea. He notes that it is of fairly frequent occurrence and claims that it is associated with syphilis in the parents. He gives as his figures, however, no definite rate of occurrence. Here it may be mentioned that the Levaditi stain done on the tissues of this specimen showed no spirochætes. On the right scapula the vertebral margin passes as a straight line from the medial termination of the spinous process to the incisura next the glenoid process. The vertical diameter of the right scapula measures 36 mm. from the tip of the cartilaginous process at the inferior angle to the superior margin near the incisura. Its transverse diameter along the base of the spinous process, near the termination of the latter, to the center of the glenoid fossa is 26 mm. The subscapular angle is 128°, the infraspinous angle is 122°, and the supraspinous angle is 110°. On the left scapula, the vertebral margin above the spinous process projects at a fairly sharp angle near its middle. The vertical diameter taken from the tip of the inferior angle to the end of the projecting point of the supraspinata is 29 mm. The horizontal diameter of the left scapula, measured similarly as the right, is 36.5 mm. The vertebral margin of the left scapula at the termination of the spinous process is elongated by a bony and cartilaginous knob, which is attached to a curved rod of bone 10 mm.

long and 2 mm. in diameter. This rod is joined at its other end to the everted arches of the vertebræ underlying it. On the left scapula the subscapular angle is 117°, the supraspinous angle is 109.5° and the infraspinous angle is 133.5°. The left scapula shows a rather interesting condition, presenting three out of four features often associated with Sprengel's deformity (congenital elevation of the shoulders). These are, according to Horwitz: (1) changed relations of the diameters to each other; (2) bending forward of the supraspinous process; (3) prolongation or rounding of superior median angle; (4) presence of exostoses and articulations with the vertebral column.

In this case the exception to the above conditions is the superior median angle, which can hardly be called prolonged. Scapular measurements of the new-born could not be found in the literature, but two supposedly normal sets were obtained from mounted skeletons belonging to the Obstetrical Department of the Johns Hopkins Hospital, and the measurements of several other scapulæ were available through the courtesy of Dr. A. H. Schultz.

TABLE 3.—*Comparison in millimeters of the scapular measurements of specimen with those of several normal new-born.*

Specimen.	Vertebral length.	Transverse diameter.	Vertical diameter.	Morphological index.
Schultz, No. 3	165	R. 24	R. 33	R. 72.7
Schultz, No. 4	151	L. 24	L. 31	L. 77.4
		R. 29	R. 38	R. 76.3
Obstetrical Department skeleton	169	L. 30	L. 38	L. 78.7
		R. 27	R. 36	R. 75.0
Do	172	L. 27	L. 35	L. 77.1
		R. 26	R. 36	R. 72.2
No. 862*a*		L. 31	L. 29	L. 106.8

Table 3 shows that the ratio of the diameters of the right scapula of 862*a* is near those of the supposedly normal bones. The left scapula, on the other hand, has the relations of its diameters reversed. The transverse diameter exceeds the vertical. Thus its morphological index is 106.8, while none of the normal indices exceeds 80. The subscapular angle on the left side is somewhat smaller than on the right. The bony articulation joining the left scapula to the vertebral column is attached in the upper third of the vertebral scapular margin as in most of the Sprengel deformity cases. Some interest is attached to this abnormal bony spicule and various suggestions have been made concerning it. The opinion seems to be generally accepted that it arises from its scapular end. Cases are recorded in which other anomalous bones are joined only to the scapula, and their occurrence substantiates this view. Case XVI in von Recklinhausen's paper is a monster very like No. 862*a*. In it there is "ein 1 cm. langer knöcherner, rippenartiger, am oberen Winkel des knorpeligen Schulterblatts articulierender Körper." Gruber gives a case found in an adult male cadaver of a "fortsatzartigen, cylindrischen Höcker an der Vorderflache des Angulus superior der Scapula." No satisfactory hypothesis has been advanced to further explain the origin of these bones. The length of the right clavicle is 39 mm. while the left clavicle measures 34 mm. and is slightly

more bent at its distal end than the right. This shortening of the clavicle on the side of the abnormal scapula is frequent in Sprengel's deformity. The hunched position of the shoulders, so prominent externally in this case, may be seen to be due to the defective cervical and upper thoracic vertebræ, which lie crumpled to half their normal length under the scapulæ, their normal relations to these bones being quite changed.

MUSCLES.

The region of abnormal musculature corresponds, as would be supposed, to the skeleton derangements. This is limited to the neighborhood of the axial skeleton, where the affected muscles are both under and intermingled with an unusually large amount of fascia. On superficial dissection, the topmost layer of muscles is well formed, except for the trapezius, which is represented similarly on the two sides by thin strap-like bands of muscle, 3 by 1 cm. The fibers run parallel with the long diameter, from the origin of the muscle, situated in fascia lying over the everted and crumpled cervical and thoracic vertebral arches, to their insertion on the acromial extremity of the clavicle, the acromion, and spine of the scapula. Those fibers which insert on the scapular spine have become folded under the others, owing to the contracted and lowered origin of all the fibers. A condition of the trapezius similar to this has been noted in a case of total rachischisis given by Kermauner, in which case, also, lordosis and marked shortening of the spine were the underlying skeletal conditions. As Kermauner says, the association of this variety of muscle and bone defect is only natural, "for, with the marked shortening of the trunk, there necessarily exists a reduction in the cranio-caudal diameter of the muscles of this region."

Upon further dissection, the condition of the underlying muscles was determined. The rhomboidei are represented bilaterally by very thin and short muscles, only 3 mm. in length. They arise from the connective tissue over the fused and everted arches of the thoracic vertebræ, and are inserted in fascia along the inferior vertebral borders of the scapulæ. (In Le Double's work a reduction in the thickness of these muscles is recorded.) The two levator scapulæ are present. They arise from the fused transverse processes of the upper cervical vertebræ and are inserted in fascia along the superior vertebral margins of the scapulæ. There is no reduction in the size of either muscle. They are directed horizontally out instead of slanting downwards as usual. This is due to the scapulæ lying directly over the cervical vertebræ. A cross-section of the left muscle at its origin is shown in figure 21. Figure 18 shows the left scapula and the rhomboideus and levator scapulæ muscles inserted in fascia which forms a sheet between the irregular projections of the vertebral margins of the bone. The abnormal spicule of bone is attached to the scapula at the median angle between the insertions of the levator scapulæ and the rhomboideus.

On each side most of the dorsal muscles consist of an irregular longitudinal bundle which extends along the sides of the vertebræ from sacrum to occiput and which sends scattered projections on to the ribs. Under this bundle in the lumbar region the quadratus lumborum and psoas muscles lie undisturbed. On the left

side this bundle is shown somewhat diagrammatically in figure 21 and labeled *sacrospinalis*. In the lumbar region it is cylindrical and measures 1.5 cm. in diameter. It grows flatter and broader as it nears the upper part of the spine, this formation being due to a state of arrested development of the sacrospinalis and short back-muscles. The early condition of dorsal musculature which it simulates is strikingly illustrated in Bardeen and Lewis's model of an 11 mm. embryo (1901), where a bundle distinct from the ventral-lateral muscles lies bilaterally in the trough formed at the sides of the vertebræ. In another model given in the same paper of a 20 mm. embryo, the bundle may still be seen lying under the connective tissue of the region, and this divided condition of the back-muscles persists normally until about the 60 mm. stage.

The serati posterior inferior are shown by projections from the dorsal bundle which on both sides cover the proximal half of the three lowest ribs. In the upper thoracic region, lying on the surface of the bundle on each side, is a thin strip of muscle near the base of the ribs. These strips extend cranio-caudally and measure 20 by 3 mm. On the left the strip lies over the third to eighth rib; on the right side it extends over the first to the sixth rib. The serati posterior superior are not identifiable.

The direction of the muscles of the anterior cervical region, as well as of those attached to the skull, is distorted with the underlying skeleton, but the muscles are well developed and not defective. Both sterno-cleido-mastoid muscles have normal origins and insertions. The two splenii arise bilaterally from fascia under the scapulæ and are inserted normally on the mastoid process under the sterno-cleido-mastoid, and posterior to this on the occipital bone. The longissimi can be traced arising from the fascia over the cervical vertebral region and inserted on the mastoid processes. The semi-spinalis capitis muscles, arising from the upper ribs near their origin, are inserted on the occipital bone and are next to the deepest layer of musculature. The latter on each side consists of short fibers, rudiments of the short neck-muscles, the recti, and obliqui. More anteriorly most of the neck muscles are recognizable. The digaster, stylohyoid, omohyoid, and sternohyoid muscles are well developed. The longus capitis and colli are represented by a few strands along the anterior surfaces of the vertebral plate. The scaleni medii and posteriores are present as flattened bands of muscle arising in this region and inserted in the first and second ribs near their bases. The scaleni anteriores are symmetrical. They arise from the lateral processes of the superior cervical vertebræ and insert on the first rib near its center. The nerve trunks of the cervical and brachial plexus pass under these muscles and are tightly bound down by them.

Of the more anterior thoracic muscles, the pectorales are not disturbed. Both serati anterior muscles are defective and differ in their defects. On the right side there is more complete development. Here slips of the muscle arising from the distal portions of the first four ribs and from the eighth to the tenth ribs converge and are inserted around the inferior angle of the scapula. A few strands of muscle on the chest wall between the pectoralis minor and the seratus are present, which might be remnants of the latter. They are shaped like half a crescent, with fibers running longitudinally, and extend from the first rib, where they are 3 mm. broad, to the fifth rib, where they are 15 mm.

On the left side the seratus anterior is very imperfect. It is represented by a thin sheet of fascia, which originates from the first three ribs and is inserted in the scapula along the vertebral margin near the medial angle. A few scattered muscle-fibers, which also probably represent remnants of the serati, arise over the fourth rib near its base and are inserted into the inferior angle of the scapula. The origin of the fascia and these muscle-fibers is shown in figure 21 by dotted lines. Some other muscles on the left chest wall, consisting of irregular projections from the dorsal bundle which covers the proximal part of the first seven ribs, may be serati fibers which remained in their embryonic position close to the axis. Fibers which probably represent intercostal muscles pushed to the outer surface of the ribs are arranged along the lower border of the fourth rib. These extend onto the lower adjoining ribs. At the outer end they are 2 mm. across and near the base of the ribs they measure 20 mm. (See fig. 21.) Three small muscle bundles are situated at the distal end of the above-mentioned fibers.

The lateral and anterior abdominal muscles are well developed. Each rectus is 7.4 cm. by 3.2 cm. The right rectus has two inscriptiones tendinæ in its upper one-third opposite the sixth and seventh ribs.

To summarize: Those muscles which have undergone most disturbance are the trapezei, the rhomboidei, the serati posteriores superiores, the serati anteriores, and the saerospinalis and short back muscles. The location of these muscular abnormalities, situated near the chief skeletal abnormalities, demonstrates still further that the pathologieal process is a rather sharply circumscribed one, limited to the neighborhood of the axis. The inclusion of the anterior serati in this group does not contradict the statement, as the early anlage of the serati is very near the axis.

The musele disturbances of "monsters" have been but little investigated or recorded. From the scattered observations at present obtainable, any correlation is impossible.

VISCERA.

On dissection, the viscera are found crowded and somewhat distorted, but, with the exception of the right lung, are well developed. The thyroid is bilobed and measures 1.5 by 1 cm., the thymus measures 6 by 2.8 by 1.1 cm. The esophagus measures 4 cm. from epiglottis to cardia. The lesser curvature of the stomach is 1 cm. and its greater curvature 8 cm. The intestines are well formed. The appendix measures 8.5 cm. The colon is much bent upon itself. Because of unskillful handling the positions asssumed by the rest of the intestinal tract were not ascertained. The pericardium, pleura, and diaphragm are intact. The heart is well developed. Sagittal section shows it cut through the right ventricle and left auricle. The valves are well formed. The ductus arteriosus is patent. The left lung is approximately normal; its lateral surface is shown in figure 23; it consists of two lobes and measures 3.9 cm. antero-posteriorly by 3.1 cm. perpendicularly by 1.7 cm. in its thickest medio-lateral diameter near the hilum. The right lung, side view of which is shown in figure 22, about equals the left in volume and is roughly a flattened cone-shape with apex directed anteriorly. Its corresponding measurements are 5.1 by 3.2 by 2.9 cm. It is formed of only one lobe. Along the margins four short fissures exist, directed toward the center; one 12 mm. in length

is situated on the posterior margin at the junction of the superior third with the middle third; on the inferior margin near its middle a similar fissure is situated, and halfway between it and the anterior end of the lung a shorter fissure 3.5 mm. long exists; on the superior margin another, 3.5 mm. in length, is present slightly anterior to the middle. These fissures are very superficial and extend for only a few millimeters on the medial surface of the lung. The relations of the bronchial tree were not determined.

The liver is flattened out horizontally and shaped like an L with the angle projecting anteriorly, the gall-bladder, which is 4.1 cm. in length, being situated on the inferior surface of the long arm of the L. The closed end of the gall-bladder lies near the tip of the angle and its long axis is directed diagonally toward the upper end of the latter. The spleen is 2.2 by 1.3 by 1 cm. The presence of the pancreas is determined histologically. It lies embedded in tissue near the vertebral column. Both kidneys and adrenals are somewhat compressed and distorted, the left much more than the right. The right kidney is somewhat flattened from side to side and at its upper end, and measures 4.5 by 1.2 by 3.5 cm. The right adrenal lies above it and measures 3 by 2 by 0.5 cm. The left kidney is bent upon itself and folded in with its closely adherent adrenal, so that together they form a rounded mass measuring 4.7 by 3.4 by 2.4 cm. The greater distortion of the left kidney and adrenal is very probably due to crowding, a result of the left-sided concave bending of the vertebral column in this region and fusion of the lower ribs on that side. The ureters and bladder are well formed. The uterus, tubes, and ovaries are well developed. Blocks of tissue of heart, kidney, liver, and adrenal were run through by the Levaditi method for spirochætes by Dr. Bullard, with negative findings. It is to be noted that the tissues had been kept in carbolic, which is not the fixation recommended for this method.

The developmental anomalies of the soft palate and the right lung are the most marked changes which have taken place in the soft tissues anterior to the vertebral column. They are both examples of arrested development and are secondary to, and probably the mechanical result of, the deformity of the vertebræ.

CRANIAL CAVITY AND CENTRAL NERVOUS SYSTEM.

The shape assumed by the cerebro-spinal cavity or subdural space is shown by figures 4, 5, 6, and 7 of the wax model. In figures 12 and 21 the model is given in its relation to the skeleton. The space consists of a shallow dome which contained the frontal and part of the parietal cerebral lobes. Below this dome a relatively slight constriction in the model denotes the enlarged foramen magnum (figs. 4, 5, 7, and 12). Under the foramen three rounded encephaloceles project posteriorly, and below these the pointed termination of the spinal canal may be seen. Situated ventral to the encephaloceles and continuous with them and with the base of the dome and the spinal canal is a blunt, wedge-shaped mass marked *w* in figures 4, 5, and 6. This portion fits into the pocket of bone formed by the thoracic and vertebral plate. With the exception of the inside of the large encephalocele, the space occupied by the central nervous system is lined with a continuous sheet of smooth dura. At the foramen magnum and in between the eminentiæ arcuatæ of the tem-

poral bones and the sella turcica it is drawn into numerous folds. On the inside of the large encephalocele the smooth dural surface changes to a tissue composed of many blood-vessels, fibrin, and extravasated blood, as described in the beginning of the paper. The falx cerebri lies well over on the left side in its anterior and middle portions; posteriorly it ends in a single fold about the center of the superior margin of the occipital bone. The tentorium cerebelli is absent. The ventral surface of the subdural space is pierced by two rows of cranial and spinal nerves. These number 43 in all, 12 cranial and 31 spinal, the latter distributed as follows: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal. Both the anterior roots and the posterior roots with their ganglia are identified. In the cervical and thoracic regions they are extremely crowded.

The arrangement of the central nervous system is very complicated. The cerebrum lies part above and part below the foramen magnum. The cerebellum lies entirely below it. The medulla and cord lie bent and crumpled ventral to the other structures below the foramen. The surface of that part of the cerebrum which lies above the foramen shows definite fissure and sulcus formation. Below the foramen a large part of the contents of the encephaloceles consists of a hemorrhagic mass and much of the surface of this is covered with caked blood, so that only in a few places can typical cerebral surface markings be identified. Sections of these regions, however, show definite though rather undifferentiated cortical lamination.

From a brief study of sections of other parts of the left cerebrum, made by Dr. Charles Bagley, the following points are determined: The cortical structure for the most part is composed of a very wide granular zone, which is characteristic of the early and undifferentiated stage of lamination. There is, however, a prominent vertical fissure lying at the junction of the middle and posterior two-thirds of the cranial dome, which can be identified as the central fissure of Rolando; the ventral termination of this fissure rests on the eminentia arcuata of the temporal bone. The cortical tissue anterior to this fissure shows a definite lamination. There is a well-defined first layer; a broad and poorly differentiated second layer; the third layer is of medium width and is filled with small pyramidal cells; the fourth layer is inconspicuous, suggesting the agranular motor type; while the fifth layer is represented by large pyramidal cells, probably Betz cells. These cells are at least three times as large as any other cells which could be found in the cortex and it is owing to their presence and to the very narrow granular layer that this area is identified as motor cortex. Immediately posterior to this fissure there is a sharp change in lamination types. The large cells are lost and the fifth layer is represented by definitely shaped pyramidal cells of not more than one-third the size of those cells designated as Betz cells. Above this layer there is present a very heavy granular layer which is quite a contrast to the narrow granular layer of the area just described. It may be said, therefore, with a fair degree of certainty, that the tissue posterior to the fissure represents sensory cortex.

From the rather limited amount of material studied the only other localization that could be determined is that the cortical tissue pushed down on the side of the wedge-shaped mass (H' in the model) represents subiculum. Here, passing from a cortex of quite deep extent, it becomes suddenly shallow and consists of a typical

first-zone layer containing few cells and a well-marked second layer with only a narrow zone of undifferentiated cortex beneath it. No calcarine type of cortex was identified, but the occipital lobe was probably included in the hemorrhagic mass in the encephaloceles, which was in such a bad state of preservation that no sections could be made.

On the right side of the cerebrum the fissure corresponding to the left central fissure of Rolando is situated slightly more anteriorly than on the left. The frontal lobes thus occupy most of the shallow vault and rest in the anterior and in the medial cranial fossæ as well, which latter normally hold the temporal lobes. The sulci of the frontal lobes are changed considerably from their normal positions. The anterior ends of the superior medial and inferior frontal sulci are bent very sharply downward. They are all situated nearer the base of the brain than usual, as if the tissue had been pulled down on the lateral surfaces. Thus the superior sulci lie halfway down the sides, with the medial and inferior sulci correspondingly below them. With the exception of part of the parietal lobes on each side, the rest of the cerebrum lies below the foramen magnum. The left-sided position of the falx cerebri allows more room in the cranial cavity proper for the right cerebral hemisphere than for the left. This results in a larger portion of the parietal lobe on the right side lying above the foramen magnum than on the left side, and correspondingly a larger amount of parietal surface lying below the foramen on the left than on the right side.

The cerebral tissue which lies below the foramen is partly contained in the large bony pocket formed by the upper vertebral plate, and partly in the middle and left encephalocele. Sulci are present over its surface, but are so distorted that they can not be identified, nor can the identity of the lobes be determined. In the contents of the middle and left sacs one can easily discern cerebral gyri, and the general histological structure of these is similar to that of the cerebral tissue situated above the foramen. In the middle sac there is a large amount of clot. The cerebral tissue which lies in the cervical and thoracic vertebral pocket is pressed out into a thin shell, and lies next the dura, being limited anteriorly by the emerging cranial nerves. On the sides and back it is continuous with the cerebral tissue lying in the cranial vault and with that pressed out into the encephaloceles.

In the interior of the brain the optic thalami may be identified, lying above the foramen. A small space representing the third ventricle, greatly compressed, lies between the thalami. Choroid plexus tissue is present. Its relations, however, to the adjoining structures could not be determined. The cerebral peduncles may be seen as flattened bundles lying central to the shell of the cerebral cortex. The optic nerves are present. The hypophysis lies embedded in the well-formed sella turcica. No other structures in this region or below can be identified until, in the pocket of bone formed by the thoracic vertebræ, the inverted floor of the fourth ventricle is recognized.

The midbrain with attached fourth nerves, the colliculi, and the aqueduct of Sylvius were not identified. The fourth nerves, however, were found at their dural exit. Judging from the position of the fourth ventricle floor, a sharp bend

with the angle directed posteriorly must have occurred in the midbrain region. At the beginning of the spinal cord a bend in the opposite direction is present. This bending of the brain stem and cord must have been in lateral outline shaped like a crudely drawn letter Z, as shown in figure 24, which is a diagram of various structures in the central nervous system near the midline. The surface of the inverted fourth ventricle floor is shaped like an isosceles triangle with its tip, which is its normal anterior end, directed backward toward the encephaloceles. The median sulcus is well defined. The tissue next to the median sulcus on both sides is slightly raised. The rest of the surface is flat. For estimating roughly the amount of distortion this fourth ventricle floor had undergone, a comparison of it with the fourth ventricle floor of three normal full-term fetuses was made. Each of the three showed a similar longitudinal ridge to be the extent of their surface markings. The main difference which this specimen showed seemed to be in a blunting of the posterior end which forms the base of the triangle already referred to.

From both lateral margins of the fourth ventricle floor cortical tissue resembling the flocculus is drawn backward, downward, and to the right, joining the cerebellar cortex contained in the right sac. It must be noted that while the flocculus is directed toward the posterior end of the specimen as a whole, it is drawn toward the end of the ventricle floor normally anterior. There is a much disturbed choroid plexus folded in with the cerebellar tissue. A fairly large amount of cerebellar tissue is present; part of this is drawn out into a sheet which is continuous with the flocculus and which passes posteriorly and to the right into the right encephalocele, where it lies next to a rounded mass of cerebellar cortex. Bands of tissue connecting the cerebellum and cerebrum probably represent the brachium conjunctivum. There are smaller flat bands of tissue passing backward near the flocculus to the rounded cerebellar cortex which may have been remnants of the inferior cerebellar peduncles. There is no pontine enlargement.

From the tissue superior to and continuous with the floor of the fourth ventricle, the third and the fifth to the twelfth paired cranial nerves pass forward to their normal exits from the subdural space. They are elongated to between 20 and 30 mm. Their origin from the brain-stem lies opposite the first thoracic vertebra. In this region the beginning of the flattened cord can be made out, which is bent double upon itself. Some interest is attached to this Z bend of the brain-stem and cord. It seems to have been brought about in this case through traction on these parts by the major portions of the central nervous system slipping through the enlarged foramen magnum. Varying degrees of such kinking have been described. The condition in its milder forms has received the name of Chiari deformity, from a case described by Chiari in which the medulla is bent back over the cord for only a short extent. In Nageli's case of cyclopia there is a marked degree of such bending associated with splitting of the cord.

Caudal to the bend as a flat band the spinal cord extends to the level of the lumbar vertebra, where it terminates in a cauda equina. From its ventral surface the spinal nerves extend into the dura. At the level of the twelfth thoracic vertebra the spur in the vertebral plate has left an indentation on the flat cord and on the right anterior third of the inverted floor of the fourth ventricle.

The central canal, as such, is absent. Throughout the extent of the cord it is changed to a flat space following the contour of the vertebral column, whose floor is the cord and whose roof is partly the same cord inverted, partly the inverted floor of the fourth ventricle, and partly cerebellar tissue.

This fragmentary description of the central nervous system leaves much to be desired. It would have been especially desirable had we been able to present a clear picture of the relationships of the meninges. The main conclusion which can be drawn from its study is that the chief disturbance here evidenced is primarily one of distortion, rather than of absence or real lack of development of nerve-tissue.

CONCLUSION

The exterior alone of such a specimen as this certainly presents striking evidence that an organism can undergo most serious disturbances and yet maintain a definite though limited growth balance; but in order to ascertain in detail exactly what constitutes the limitations of this equilibrium more intensive study is necessary. A rather interesting series of anomalies is the result of such a study in this case. It may be noted that these anomalies are centered about the axis. The bony parts, the central nervous system, certain adjacent muscles, and overlying areas of integument share profoundly in this disturbance. Subsidiary disturbances of development are evidenced in a split soft palate and a one-lobed right lung. These facts, in addition to supplying a clearer knowledge regarding the individual specimen, contribute their small share in providing data for the better understanding of certain general problems of development. Classifications and analyses included in such subjects as osteology, myology, and organology can not be regarded as complete until they contain a comprehensive picture of teratological phenomena. This is almost entirely lacking at present. The teratological material has been so scanty that any satisfactory correlation of it has been impossible.

Up to fairly recent times teratology was considered an isolated science; it was thought that the laws applying to most natural phenomena were not applicable to its conditions, that it could not learn from or contribute to the normal sciences. Studies of the past half century have entirely reversed this view. Teratology today has for its basis the same fundamental sciences of chemistry, biology, and physics as has those sciences whose subject-matter deals with normal phenomena. It is constantly learning from these latter sciences, and in turn has been able to contribute suggestions on points of analysis or exposition regarding puzzling phases of normal development.

The necessity of furthering our knowledge regarding the etiological factors of specific abnormal conditions has been considered. Material at such an advanced stage of development as this specimen can contribute but little along this line. We can not determine by means of it the primary defect, nor again, except in a very general way, a chronological picture of the early processes. We must turn to embryological material and to other than morphological methods to obtain such knowledge.

BIBLIOGRAPHY.

Aeby, Chr., 1879. Die Altersverschiedenheiten der menschlichen Wirbelsäule. Arch. f. Anat. u. Physiol., Anat. Abt., pp. 95, 109.

Baldwin, W. M., 1915. The action of ultra-violet rays upon the frog's egg. Anat. Record, ix, 365–381.

Ballantyne, J. W., 1904. The embryo. Antenatal pathology and hygiene, Edinburgh, 272, 285.

Bardeen, C. R., and W. H. Lewis, 1901. Development of the limbs, body-wall, and back in man. Amer. Journ. Anat., i, 1–35.

Bayer, C., 1889, 1890, 1892. Zur Chirurgie der Rückenmarksbrüche. Prager med. Wochenschr., xiv, 227–230; xv, 48; xvii, 317, 332, 345.

Bettmann, 1912. Die Missbildung der Haut. *In* E. Schwalbe, Die Morphologie der Missbildungen, III Teil, VII Lieferung, 2. Abt., 7. Kap. Jena.

Binder, 1899. Das Morelsche Ohr. Arch. f. Psychiat., xx, 514–564.

Brodman, K., 1910. Feinere Anatomie des Grosshirns, p. 206. *In* M. Lewandowsky, Handbuch der Neurologie. Erster Teil, ii. Anatomie. Berlin.

Chiari, 1891. Veränderungen des Kleinhirns in Folge von Hydrocephalie des Grosshirns. Deutsche med. Wochenschr., xvii, 1172–1175.

Ernst, Paul, 1909. Missbildungen des Nervensystems. I. Störungen des Verschlusses in der hinteren Mittellinie des Körpers. *In* E. Schwalbe, Morphologie der Missbildungen, III Teil, I Lieferung, II Abt., II Kap., I. p. 72.

———, 1909. Missbildungen des Nervensystems, X. Entwickelungsstörungen des Kleinhirns. *In* E. Schwalbe, Morphologie der Missbildungen, III Teil, I Lieferung, II Abt., II 2. Kap., (X) p. 223

Gillaspie, C., L. I. Miller, and M. Baskin, 1916. Anomalies in lobation of lungs. Anat. Rec., xi, 65-75.

Good, J. Percy, 1912. Spina bifida in the neck region of a ferret embryo, 8 mm. Journ. Anat. and Physiol., xlvi, 391–399.

Graves, Wm. W., Scapula scaphoidea, eine häufig vorkommende Anomalie des Schulterblattes. Ihr. Zusammenhang mit Syphilis in der Ascendens. Med. Klin., 1911, No. 8, p. 298.

Gruber, Wenzel, 1872. Ueber einen fortsatzartigen cylindrischen Höcker an der Vorderfläche des Angulus superior der Scapula. Virchow's Arch. f. path. Anat., lvi, 425.

Grünberg, Karl, 1913. Die Missbildungen des Kopfes. Die Spalte des weichen Gaumens. In E. Schwalbe, Die Morphologie der Missbildungen. III Teil, IX Lieferung, I Abt. 4. Kap.

Gudden, H., 1898. Fall von Knickung der Medulla oblongata und Theilung des Rückenmarks. Arch. f. Psychiat., XXX, 866-876.

Hertwig, O., 1892. Urmund und Spina bifida. Arch. f. mikr. Anat., xxxix, 353–530.

Hildebrand, 1893. Ueber Spina bifida. Verhandl. d. deutschen Gesellsch. f. Chir., xxii, 41–43.

Horwitz, A. E., 1908. Congenital elevation of the scapula, Sprengel's Deformity. Amer. Journ. Orthop. Surg., vi, 260–311.

Kermauner, 1909. Missbildungen des Rumpfes. *In* E. Schwalbe, Morphologie der Missbildungen, III Teil, I Lieferung, I Abt., 3. Kap., p. 86.

Koch, Wilhelm, 1881. Mittheilungen über Fragen der wissenschaftlichen Medicin. 1. Heft. Beiträge zur Lehre von der Spina bifida. Kassel.

Lewis, H. F., 1897. Iniencephalus. Amer. Journ. Obst., xxxv, 11–53.

Lewis, W. H. (See Bardeen and Lewis.)

Lillie, Ralph D., 1917. Variations of canalis hypoglossi. Anat. Record, xiii, 131-144.

Macklin, C. C., 1914. The skull of a human fetus of 40 mm. Amer. Journ. Anat., xvi, 317, 387.

Mall, F. P., 1908. A study of the causes underlying the origin of human monsters. Wistar Institute of Anatomy and Biology. Philadelphia. (Journ. Morphol., xix, 1–367.)

Marchand, 1899. Spina bifida. Missbildungen. Real Encyclopädie der gesammten Heilkunde, von A. Eulenburg. 3 Aufl., 1899, xxii, 578–601.

Martin, Rudolf, 1914. Lehrbuch der Anthropologie. Jena.

Marx, Hermann, 1911. Die Missbildungen des Ohres. *In* E. Schwalbe, Morphologie der Missbildungen, III Teil, V Lieferung, II Abt., 6. Kap. Jena.

Muscatello, 1894. Ueber die angeborenen Spalten des Schädels und der Wirbelsäule. Arch. f. klin. Chir., xlvii, 162, 257.

Naegeli, O., 1897. Ueber eine neue mit Cyclopie verknüpfte Missbildung des Centralnervensystems. Arch. f. Entwickelungsmechanik, v, 168–218.

von Recklinghausen, F., 1886. Untersuchung über die Spina bifida. Virchow's Arch. f. path. Anat., cv., 243, 373.

Schneider, 1912. Die Missbildungen der Atmungsorgane. *In* E. Schwalbe; Die Morphologie der Missbildungen, III Teil, VIII Lieferung, II Abt., 8. Kap.

Spietschka, T., 1894. Hautveränderung bei Spina bifida. Prager med. Wochenschr., xix, 117, 131.

Sprengel, 1891. Die angeborene Verschiebung des Schulterblattes nach oben. Arch. f. klin. Chir., xlii, 545–549.

Streeter, G. L., 1903. Anatomy of floor of the fourth ventricle. Amer. Journ. Anat., ii, 299–313.

———, 1915. Development of the venous sinuses of the dura mater in the human embryo. Amer. Journ. Anat., xviii, 145–178.

Tillmanns, H., 1905. Die Verletzungen und chirurgischen Krankheiten des Beckens. Deutsche Chirurgie, Lieferung 62 *a*, p. 497.

Weed, Lewis H., 1916. The development of the cerebro-spinal spaces in pig and in man. Contributions to Embryology, v. 5, No. 14. Carnegie Inst. Wash. Pub. No. 225.

EXPLANATION OF PLATES.

ABBREVIATIONS.

anth.,	anthelix.
antitr.,	antitragus.
b. occ.,	basioccipitales.
c. i.,	first coccygeal segment.
c. c. 7,	costal cartilage of seventh rib.
c. eq.,	cauda equina.
cer.,	cerebrum.
cereb.,	cerebellum.
cr. 2,	second cranial nerve.
cr. 5,	fifth cranial nerve.
dep. em.,	depression made by eminentia arcuata of temporal bone.
epist.,	episternum.
eust. t.,	eustachian-tube orifice.
exos.,	exostosis.
falx.,	falx cerebri.
fl. IV,	floor of fourth ventricle.
for. mag.,	foramen magnum.
l. 1,	first lumbar segment.
l. 2,	second lumbar segment
l. 1 rib,	left first rib.
l. en.,	left encephalocele.
l. pars lat.,	left pars lateralis.
mid. en.,	middle encephalocele.
m. lat. dors.,	m. latissimus dorsi (origin).
m. lev. scap.,	m. levator scapulæ (cross-section, fig. 17, insertion fig. 14).
m. obl. ext.,	m. obliquus externus abdominis (origin).
m. pect. maj.,	m. pectoralis major (origin).
m. pect. min.,	m. pectoralis minor (origin).
m. quad. lumb.,	m. quadratus lumborum.
m. rect.,	m. rectus abdominis (origin).
m. rhomb.,	m. rhomboideus (insertion).
m. sacrospin,	m. sacrospinalis (insertion).
m. ser. ant.,	m. seratus anterior (insertion).
m. ser. post. inf.,	m. seratus posterior inferior.
naev.,	nævus.
r. 1 (rib),	right first rib.
r. hyp.,	right hypoglossal canal.
rt. en.,	right encephalocele.
s. 1,	first sacral segment.
sp. 1,	first spinal nerve.
sq. occ.,	squama occipitalis.
st.,	sternum.
tr.,	tragus.
uv.,	uvula.
v.,	vertex.
v. pl.,	vertebral plate.
w.,	central nervous system occupying bony vertebral pocket.
x.,	anomalous bone and its insertion.
xyph.,	xyphoid.
iii.,	third ventricle.
*	absence of soft palate.

Plate 1.

Fig. 1. Right lateral view of specimen shows extreme dorsal flexion with vertex level with shoulders. Middle and right encephaloceles show in this view. The distorted right ear here seen is drawn in detail in figure 8. ($\times \frac{1}{4}$)

Fig. 2. Superior view of the specimen looks directly at the face. Measurements given in table 1. Transversely across the forehead at the hair line an artefact puckering extends horizontally for 2 cm. ($\times \frac{1}{4}$).

Fig. 3. The dorsal view shows the shortened trunk, superior surface of head, and encephaloceles. ($\times \frac{1}{4}$).

Fig. 4. Right lateral surface of subdural cast, showing middle and right encephalocele. ($\times \frac{1}{4}$).

Fig. 5. Left lateral surface of subdural cast, showing middle and left encephalocele. ($\times \frac{1}{4}$).

Fig. 6. Ventral surface of subdural cast. ($\times \frac{1}{4}$)

Fig. 7. Dorsal view of subdural cast. The falx is seen to be to the left of midline. ($\times \frac{1}{4}$).

Plate 2.

Fig. 8. Sketch of right ear (natural size), showing the anthelix unusually prominent. The tragus lies relatively higher than normal, over rather than horizontally opposite the antitragus. The whole ear very narrow.

Fig. 9. Sagittal section. Main outlines were geometrically projected and detail drawn free-hand. The viscera retain approximately their normal position. Absence of the soft palate is shown. The tip of the tongue lies over the left anlage of the split uvula. The vertebral column is bent and shortened and irregularly fused in its upper part. The arches of all the vertebræ are lacking. A fibrous band lies over the upper sacral vertebra, joining the opposing defective arches in that region and forming a short spinal canal. The section passes to the left of the sella turcica. The falx cerebri is seen well over on left side. The outline of the central nervous system, as is here shown, is used reversed for a diagram in figure 24. The section passes near the median margin of the left sac. ($\times \frac{1}{2}$).

Fig. 9*a*. Gives left side of bilateral anlage of uvula and orifice of eustachian tube. (Natural size).

Fig. 10. Shows a dorsal view of the mounted skeleton, with scapulæ in place. Varying degrees of gaping vertebral arches are shown at different levels of the spinal column. In the cervical and thoracic regions defective vertebral arches are fused together and markedly everted. In the upper lumbar region they are individually distinct, but still widely everted, while in the lower lumbar and sacral regions they are distinct and bent toward one another. The lumbar transverse processes and the sacral lateral processes are well developed and the coccyx of four segments is seen bent well to the left. In the lower thoracic region a cartilaginous spur projects dorsalwards from the vertebral bodies. All the thoracic and cervical vertebral bodies are fused together in a single plate. A slight lateral bending in this plate is present. The foveal surfaces of the atlas face the reader. The intervertebral foramina show large spaces in the lumbar region, which are a sharp contrast to the tiny areas of the contracted thoracic intervertebral foramina. On the right, the rough

surface of the tip of the first lumbar arch is shown, which joins the occiput; and on the left, the second lumbar arch, which does the same. Crowding of the base of the ribs may be seen, including the first to the sixth on the right and the fifth to the ninth on the left. The sternum is considerably to the left of the midline. A persistent episternum is present as a small cartilaginous knob, surrounding the manubrium. The irregular vertebral and superior margins of the scapulæ are shown. On the left side the spicule of bone passes from the thoracic and cervical arches to the scapula. (Natural size).

FIG. 11. The superior view of the thoracic skeleton and the anterior surface of the cervical vertebral plate and of the occiput. In the cervical part, no vertebral bodies are distinct, but irregular radicular, and transverse processes project laterally from the central plate. The abnormal spicule of bone on the left side may be seen passing from the fused transverse processes to the left scapula. This view shows how the foveal surfaces of the atlas are shifted to the right in relation to their underlying transverse processes. The right fovea almost overlies the tip of the right transverse process, while the left fovea leaves the left transverse process uncovered. The left transverse process is bent up and joins the pars lateralis, thus forming a rather large foramen. The anterior surface of the occiput shows an asymmetrical oval outline pierced by a foramen, in its center. The double exit of the right hypoglossal canal shows. The irregular superior margins of the scapulæ are seen. The episternum and the aborted second rib are demonstrated. (Natural size).

PLATE 3.

FIG. 12. The left-hand view of the axial skeleton with subdural cast in place and median outline of specimen given This figure shows the extreme dorsoflexion of the vertebral column. The occiput is in position and its squamosal junction on the left with the second lumbar arch is shown. The origin of the spicule of bone which projects out from the transverse process in the thoracic region is visible. The crowding and irregular arrangement of the fifth to the ninth ribs is shown. ($\times\frac{1}{2}$).

FIG. 13. The superior view of the occipital bone shown with its enlarged foramen magnum. A normal-sized foramen is designated by a dotted line. The left jugular process is prominent when compared with the right, which seems to have been twisted over to the side. The anterior outlet to the right hypoglossal canal is shown with the tiny rod of bone which divides the exit of the hypoglossal foramen on the right side immediately under it.

FIG. 14. The inferior surface of the occipital bone shows the large foramen. On the squamosal surface the exostoses which join the lumbar vertebræ show. On the partes laterales the condylar surfaces and on the left side the cartilaginous process which joins the transverse process of the atlas may be seen. The notched basal margin of the basiocciput also is visible. (Natural size).

FIG. 15. This shows schematically the ventral surface of the thoracic vertebral plate with pairing of the origin of the seventh and eighth and ninth and tenth ribs on the right side, and of the first and second, and the third and fourth on the left.

FIG. 16. This shows schematically the sternum with six costal cartilage attachments on each side. The last attachment on both sides is that of the seventh rib. The discrepancy occurs through the second rib becoming aborted on the right side, and the sixth being aborted on the left side. There are four centers of ossification on the midline of the sternum. The upper two are opposite the first costal cartilage and resemble an exclamation mark. The lower two are oval (4 by 3 mm.), with long diameter perpendicular. One is about at the middle point of the sternum and the other 1 cm. below it. An episternum surmounts the sternum and the xyphoid process projects at its inferior end.

PLATE 4.

FIG. 17. Dorsal superior view of a normal left scapula of a new-born.

FIG. 18. Same view of left scapula of specimen 862*a* shows the irregular vertebral and superior margins with the abnormal spicule of bone attached. It also shows the sheets of fascia attached to the vertebral and median margins of the scapula and the insertions of the rhomboideus and levator scapulæ muscles on this fascia. (Natural size).

FIG. 19. Dorsal superior view of a normal right scapula of a new-born.

FIG. 20. Same view of right scapula of this specimen, showing irregular vertebral margin. (Natural size).

FIG. 21. Diagram of left thoracic and deep dorsal musculature on the left side of the mounted axial skeleton. The occiput and model of cerebro-spinal cavity are in place. The median outline of the specimen is also given in relation to these structures. Those muscles approximately normal are either sectioned or only drawn at their origin or insertion. They are the m. pectoralis major and minor, the rectus, the external oblique, the latissimus dorsi, the quadratus lumborum, and the levator scapulæ. The abnormal muscles are shown entire, except for the serratus anterior, whose origin is indicated by broken lines. The largest mass of abnormal muscles consists of a longitudinal bundle extending from the sacrum to the occiput and labeled m. sacrospin. From about the center of this bundle the serratus posterior inferior projects onto the lower three ribs. The muscles at the upper end of the bundle are quite irregular. Along the fourth and fifth ribs a mass of muscle extends nearly to their costal cartilages. At the distal termination of these fibers lie several small irregularly placed bundles. In the upper thoracic region is a narrow band of muscle overlying the others. ($\times\frac{1}{2}$).

FIG. 22. Lateral view of abnormal right lung formed of but one lobe. ($\times\frac{1}{4}$).

FIG. 23. Lateral view of normal two-lobed left lung. ($\times\frac{1}{4}$).

FIG. 24. Diagram of those structures of the central nervous system which lie near the midline and which can be identified. The outline of the subdural space used was obtained from the sagittal section. Posteriorly this passes near to the median margin of the left encephalocele. The cerebrum designated by a dotted field is shown protruding below the foramen magnum into the encephalocele. A small portion of the cerebellum, represented by line-hatching, is seen to lie very much flattened on top of the cord. The brain-stem and cord, much bent, are shown in solid black. Those cranial nerves which were identified are shown by lines. Only the first spinal nerve is shown. The floor of the fourth ventricle lies inverted on top of a flat cord.


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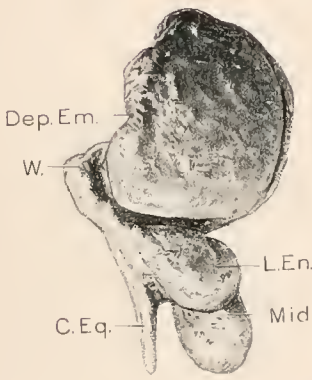
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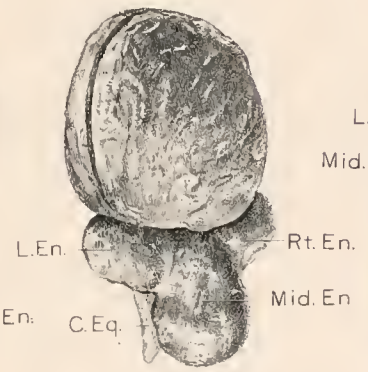
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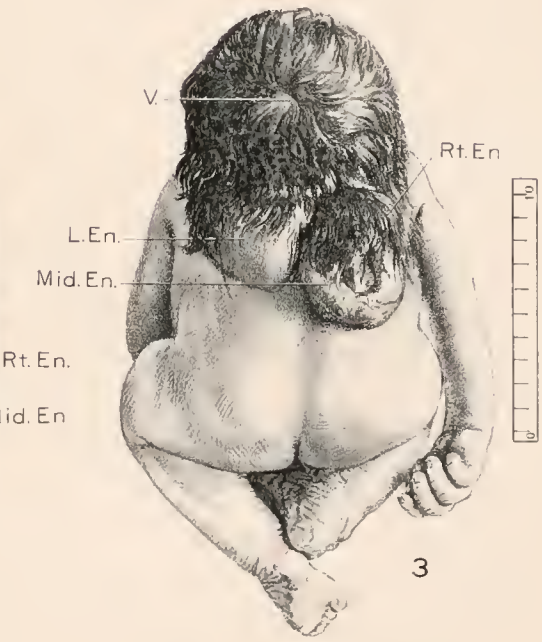
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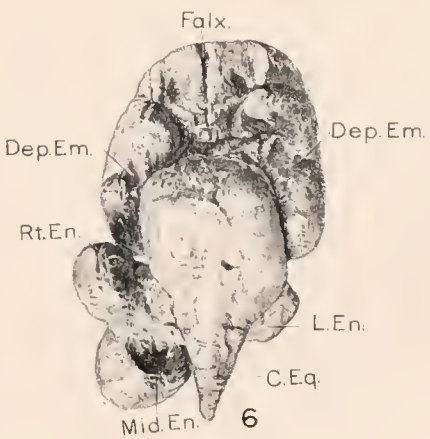
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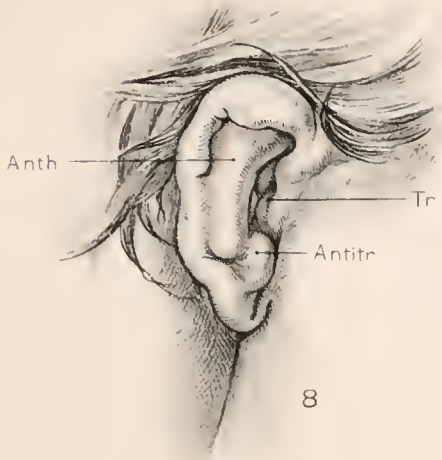
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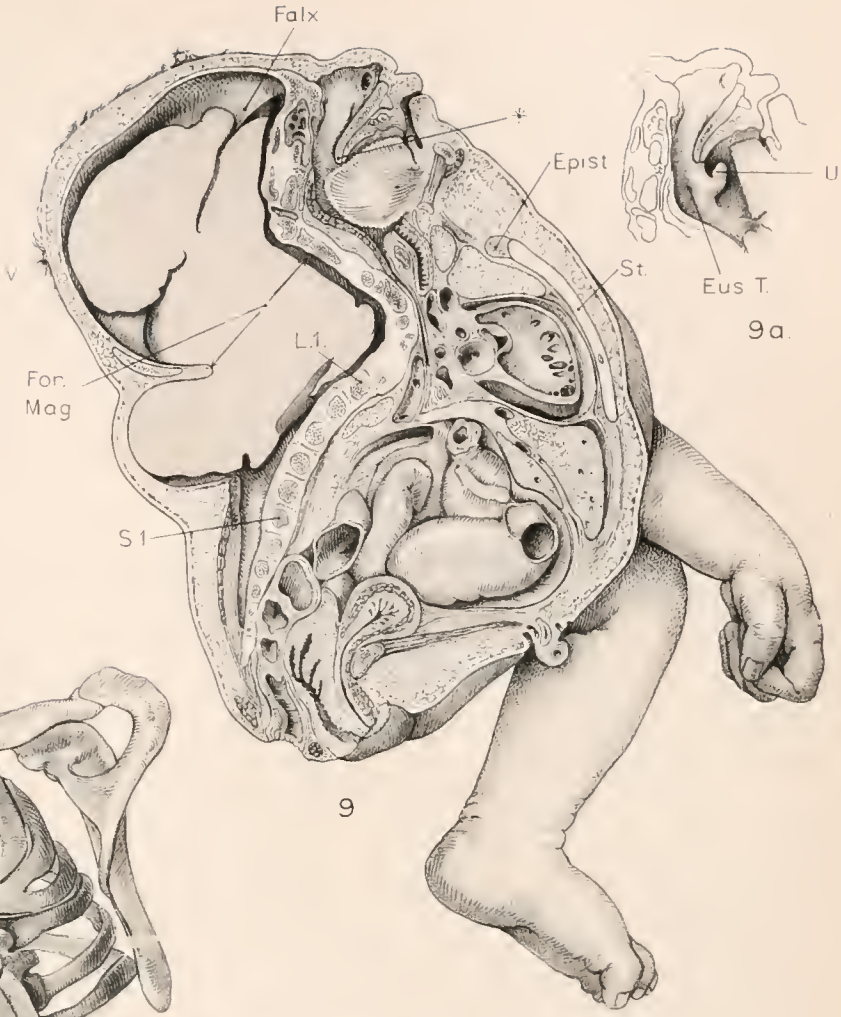
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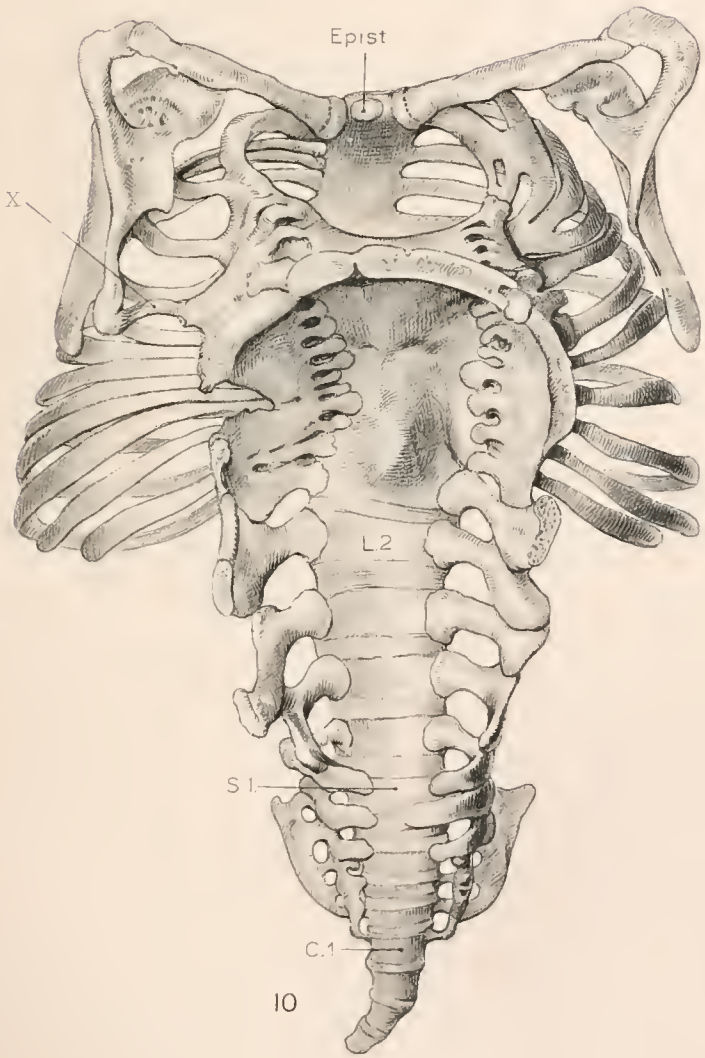
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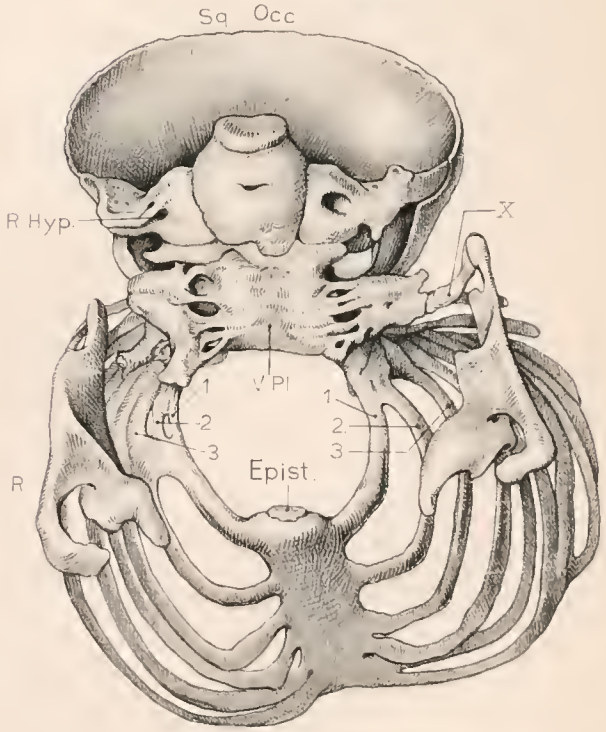
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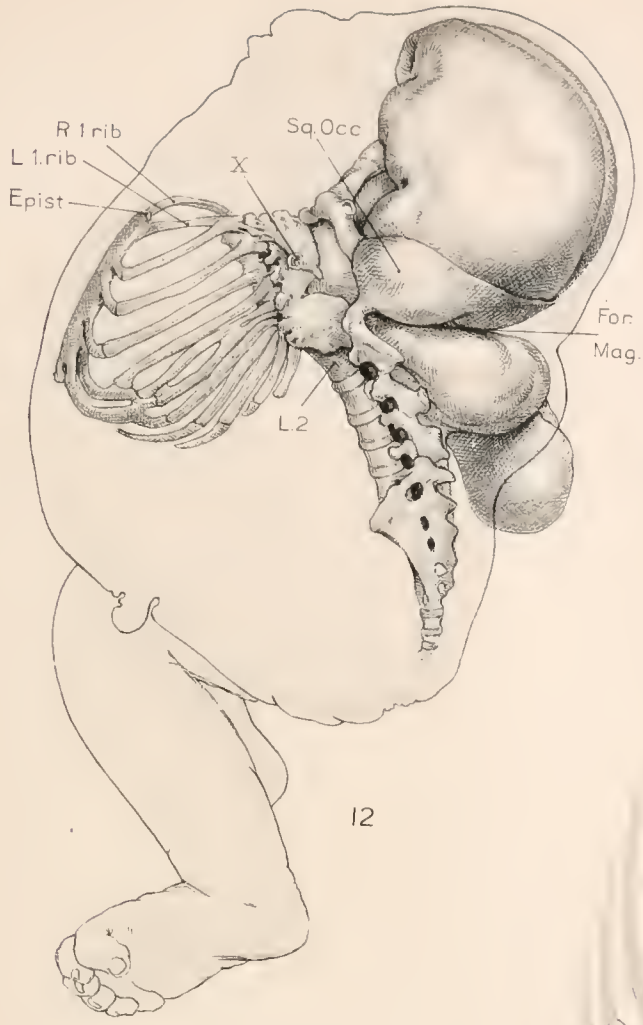

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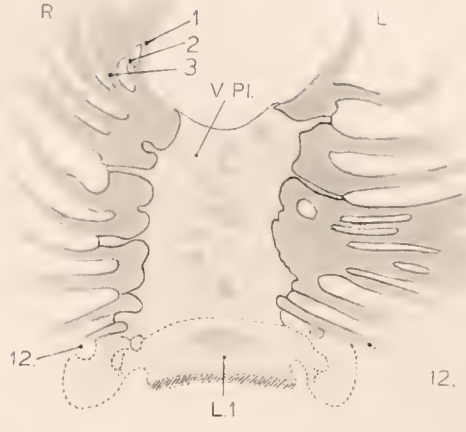

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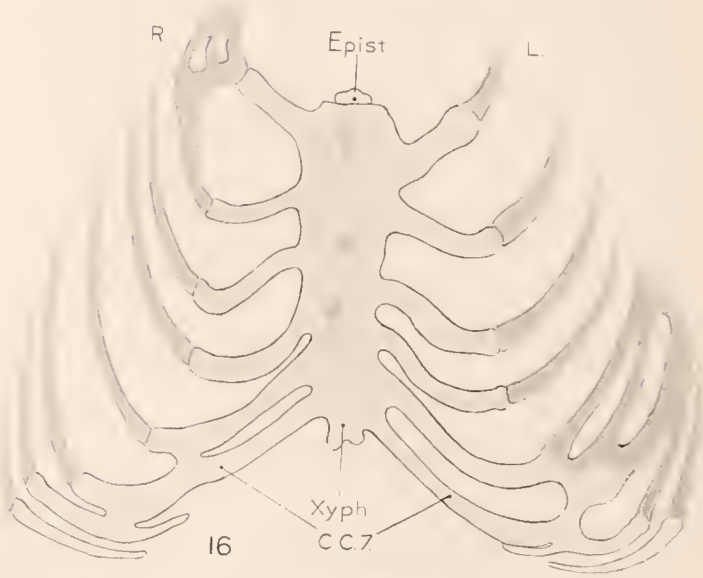

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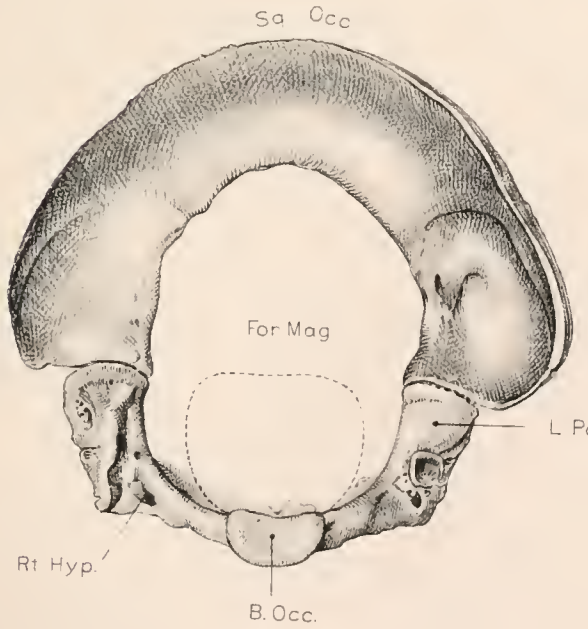

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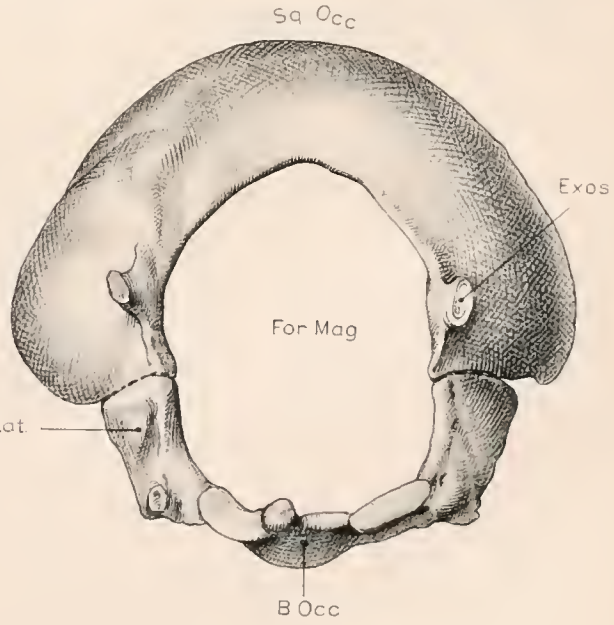

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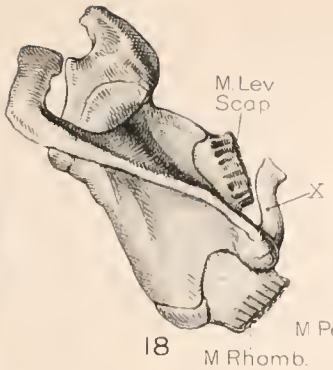

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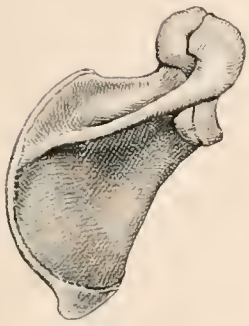

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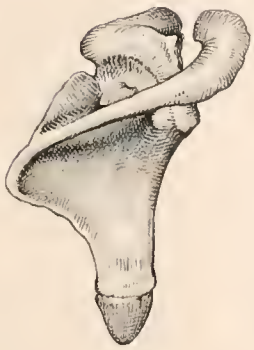

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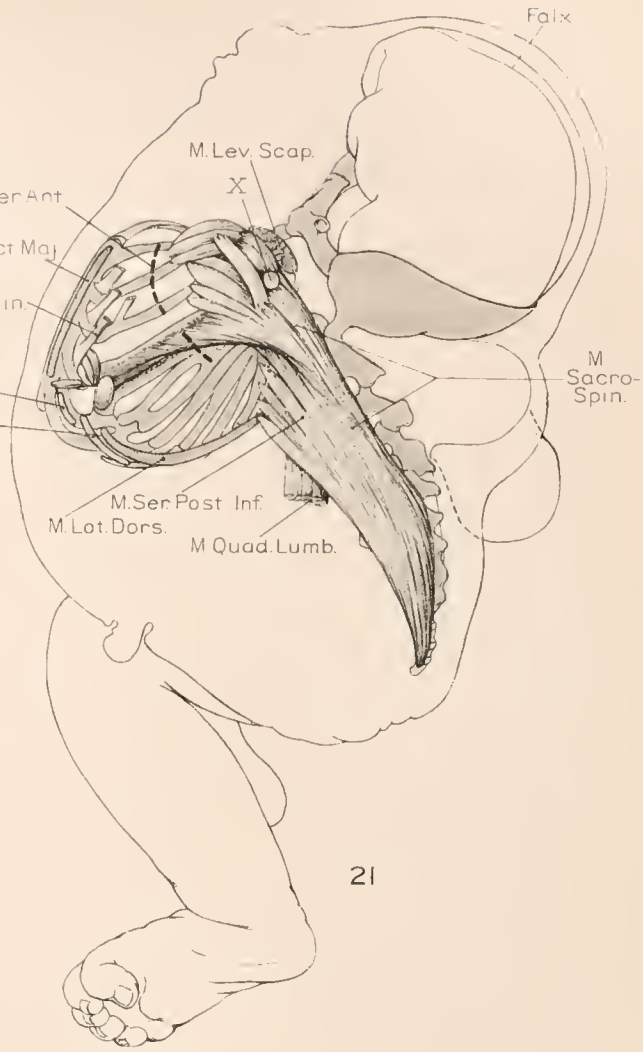

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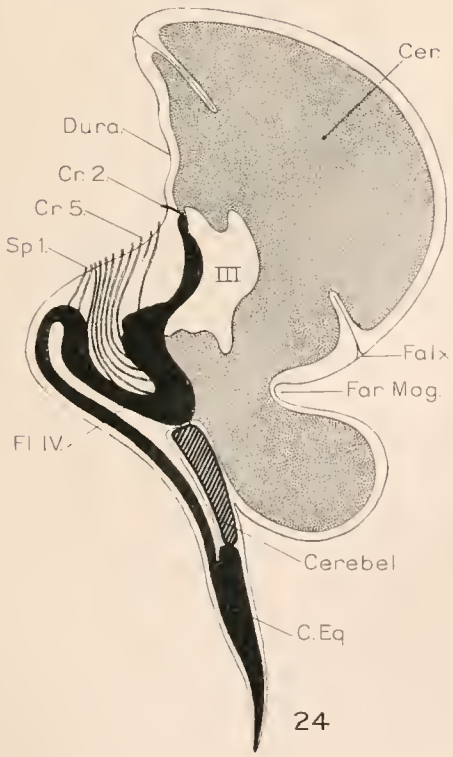

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CONTRIBUTIONS TO EMBRYOLOGY, No. 23.

A HUMAN EMBRYO BEFORE THE APPEARANCE OF THE MYOTOMES.

By N. William Ingalls.

With four plates and five text-figures.

A HUMAN EMBRYO BEFORE THE APPEARANCE OF THE MYOTOMES.

By N. William Ingalls.

The specimen which forms the subject of this paper came into my possession some time ago through the kindness of Dr. E. Peterka, of Cleveland. In the collections of embryology and teratology of the Department of Anatomy of Western Reserve University it is listed as embryo No. 1. On account of the very interesting and important stage of human development which it illustrates, a detailed investigation of its more essential features, especially as regards the embryo proper, has been undertaken. The extra-embryonic structures, chorion, body-stalk, and yolk-sac, and the evidence they offer on early blood and blood-vessel formation, will not be dealt with in detail at this time.

The intact ovum, when it came into my hands, had been for about a month in alcohol of unknown strength, but was, on account of its small size, quite well preserved. The following brief history accompanied the specimen:

April 2. Intercourse (also about two weeks before?).
April 8. Period expected; regular 24 to 26 days.
April 14. Bleeding commenced, gradually increasing.
April 17. Ovum cast off.

Before entering upon a discussion of the anatomical findings, something may be said as to the probable age of the specimen. Following the example of Bryce and Teacher (1908), which has been adopted so frequently, one can set up a similar table for the embryo in question:

Dimensions in millimeters.			Days elapsed from—			Age in days.	Remarks.
Ovum		Embryo (amniotic cavity).	Last period.		Lapsed period.		
External.	Internal.		Beginning.	End.	Beginning.		
9.1×8.2×6−6.5	ca. 1−1.5 less	ca. 2.0	34	(?)	9	(17 to 18)	Abortion 15 (29) days after intercourse.

The estimated age of 17 to 18 days was put in parenthesis in the above table because we could not bring ourselves to look upon it with any very great degree of confidence. The figures were obtained by comparison with embryos which were obviously in a stage of development either more or less advanced and by reference to the recent estimates of Triepel (1914) and Grosser (1914). Triepel's suggestion of subtracting about 18 days from 34 in this case would give an age of about 16 days. Embryo No. 1 is far in advance of both that described by Fetzer (1910) and the v. Herff embryo of Graf Spee (1896), the ages of which have been given as 15 and 17 to 18 days respectively. On the other hand, it is distinctly less advanced than Frassi's

(1907) specimen, the age of which is estimated at (18) 19 days. The embryo Kl. 13 of Grosser (1913) is strikingly like our own, but we think a trifle more developed. Grosser gives the age as 19 days. A very similar stage of development is represented by the recent embryo of Strahl (1916), but concerning which there are no data as to age.

If one again adopt the method of Bryce and Teacher, it is possible to determine when, as regards the menstrual cycle, fertilization took place. The duration of the menstrual cycle in this case may be taken as 25 days—regular 24 to 26—and if we let the age of the embryo be 18 days, then fertilization occurred on the seventeenth day of the previous menstrual month. Such a date would harmonize very well with the findings of Fränkel on ovulation, as interpreted by Grosser. The time thus assigned for fertilization could easily be pushed still farther toward the beginning of the menstrual month by either supposing that the embryo is more than 18 days old or that development was arrested by the hemorrhage some time before abortion occurred. As regards the last point, we see no reason to suppose that development stopped very long before the ovum was expelled. Fertilization on the seventeenth day of the menstrual cycle would mean that the intercourse of April 2 could not be considered in computing the age, since it falls on the twentieth of the cycle, 15 days prior to the abortion.

The history of intercourse two weeks before the one just noted is, however, subject to doubt; it would have occurred on the sixth day of the cycle. This would have called for a rather protracted sojourn of the spermatozoa within the tube—namely, 11 days, a period over which they are doubtless quite capable of retaining their fertilizing power. The sixth day of the cycle would fall about the beginning of the so-called period of œstrus, and, in view of the reputed increase in libido at this time and of certain obstetrical experiences, one may suppose that not only is this a favorable time for insemination, but also favorable to a prolonged stay of the spermatozoa within the tubes.

The arrival of the ovum in the uterus and the date of its implantation are dependent upon the unknown factor of the time consumed in traversing the tube. Grosser points out that this may vary, depending upon tubal (menstrual) conditions, and he is inclined to raise the estimate of 10 days, given by himself and Triepel, to 14 days or even more. In either case, implantation would have occurred after the beginning of the lapsed period, and some influence other than that of the actual ovum upon the uterine mucosa would have to be invoked to inhibit the impending menstruation. Such an influence, as is well known, has been sought in the tiny ovum within the tube, acting alone or in conjunction with the newly formed corpus luteum. Assuming 10 days as the period of migration (7 days in the table of Bryce-Teacher), implantation would have occurred in our specimen on the second day of the cycle. It would therefore have found a mucous membrane especially adapted to its nutritional needs, possibly thus accounting for its large size as compared with the stage of development; but, on the other hand, the inhibitive action upon this same mucosa, from whatever source, may have come too late to save it, as seems also to have been the case with the Bryce-Teacher ovum.

It is not the purpose of this paper to enter into any discussion of the relations between ovulation and menstruation, the passage of the ovum along the tube, or other mooted questions which may have a bearing on the age and development of embryos, but a few words might be said regarding what seem to us to be certain aspects of this subject. Of the three cardinal embryonic features so often quoted, viz, age, size, and stage of development, only the last has any great practical importance, and even here there is more or less variation in different parts of the embryonic body. The fact that age, size, and development by no means run parallel has been pointed out very clearly by Mall (1914; *cf.* also Rabl, 1915), and indeed there is no reason to expect that they would be exactly comparable. It seems to us that with ova of the same age, dating from the time of fertilization, discrepancies in their size and development may, and not without reason, be assigned to different environmental factors. Tubal conditions during migration, varying at different times in the menstrual cycle, might play a certain rôle, resulting in more or less rapid progress along the tube, accelerating or retarding development. Conditions in the uterus at the time of implantation, premenstrual, menstrual, or postmenstrual, etc., time of ovulation, or conceivably the actual size or potentiality of the ripe unfertilized ovum and other unknown or unappreciated factors, might bring about the variations so often observed. The relative independence of age, size, and degree of development is most strikingly evident in the case of pathological ova.

The variation in size at the same developmental stage is especially marked in the case of embryo No. 1 (see page 116). The chorionic vesicle is roughly of the same dimensions as that of the embryo Kl. 13 of Grosser or of the embryo of Eternod (1898), while the blastoderm of No. 1 is, as well as can be determined, about twice as long as in Grosser's case—but of about the same stage of development and a half longer than in Eternod's—but far less advanced. In other words, the size of the vesicle is fairly commensurate with the assigned age, while the embryo is disproportionately large, both as regards the chorionic sac and the estimated age; it is, in addition, very large, considering the degree of development. The opposite disproportion between the sac and contained embryo is quite characteristic of pathological ova, and it may be that we are dealing here with the results of some subtle influence which has stimulated the growth of the embryo proper without, however, having disturbed unduly its orderly development or brought down the balance on the pathological side. A retardation in development but not in growth might account for observed conditions. In the face of the extensive literature on the early chapters of human development we can not claim to present a typical embryo of the middle of the third week, but simply a normal specimen of about that age. It is difficult enough to find one's way among the unnumbered variations of adult morphology, but as regards the embryo we have hardly scratched the surface. It would not be surprising if we had before us in this specimen one of those examples of embryonic variation which are so abundantly present later on (tail, pronephros, milk-ridge, fifth aortic arch, etc.). For obvious reasons these individual variations become more plentiful as development proceeds, but at no time need they occasion any surprise and always may they be ranged under the same rubric.

One carries away from the perusal of the literature bearing on the age of young ova—the relations between ovulation, menstruation, fertilization, implantation, etc.—the impression that the actual age of a normal embryo has a value, for purposes of classification at least, more apparent than real if not in large measure fictitious, and the more so because this assigned age can be only a more or less defensible approximation. Complicating the more general factors touched upon above are the individual variations and peculiarities, pathological states it may be of the maternal organism if not also of the future ovum, temporary bodily or seasonal conditions and the like, not to mention possible paternal influences, a variety of factors which it is difficult or impossible to evaluate, and we are imperceptibly carried into problems of fecundity, absolute and relative sterility, and other clinical, racial, and sociological questions. In the end one can appreciate the perplexity of Hyrtl when he wrote long ago in his characteristic vein: "So weit wäre nun Alles recht. Nur begreifft man dabei nicht, warum die Frauen nicht fortwährend schwanger sind, und aus dem Schwangersein ihr Lebelang nicht herauskommen."

The entire specimen was stained in bulk with hematoxylin, and after sectioning at 10 microns was counterstained with eosin-safranin. The plane of section, which it was supposed would be transverse to the embryo, the interior of the vesicle having been examined somewhat before embedding, came out quite obliquely, as can be seen in the various text-figures. While the staining reactions are not always what could be desired, still there is no doubt that the essential features have been preserved. Occasional mitoses are in evidence, as will be noted later.

1. THE CHORIONIC VESICLE, GROSS.

The following account is taken from our notes made soon after receiving the specimen. The intact vesicle (plate 1, figs. 1 and 2) is quite regularly formed and distinctly flattened; the surface showing the circular area of free villi is slightly more convex than the opposite. The form tends to be roughly quadrangular with the corners rounded off. To the touch the vesicle feels quite firm and resistant. Measured under a magnification of 5 diameters, the ovum shows the following dimensions: length 9.1 mm., breadth 8.2 mm., thickness 6 to 6.5 mm. The internal measurements are from 1 to 1.5 mm. less.

One surface of the ovum presents a large, sharply defined area of free chorion and its villi, situated at one end and extending about to the middle. These villi vary greatly in size and shape. They may assume the form of long, slender processes or of thick, broad, irregular masses, often in clumps together and leaving a few small areas free. There are a few straw-colored areas as from blood-stains. The remainder of this surface of the ovum is smooth and varies in color from a straw through a red (fresh meat) to almost a purple.

The opposite side of the ovum is much smoother, covered partly by a much thinner layer of maternal tissue through which project more or less freely the villi of the chorion. These villi appear to be rather more pointed and slender than those previously noted, resembling papillæ filiformes. There seems to be no part of the sac which does not possess villi.

Upon making an incision along one side of the sac to facilitate embedding, a large cavity is found into which projects the embryonic anlage attached to the side showing the free villi. Regarding the embryo proper nothing more than a small, whitish, globular mass (yolk-sac) can be made out for fear of injuring the embryonic structures. At this time there were seen a few minute but distinct strands traversing the cavity (exocœlom) and connecting the inner surface of the vesicle with the yolk-sac. Traction upon the margins of the opening in the vesicle could be seen to have a very distinct effect upon the yolk-sac on account of the attachment of the above-mentioned filaments.

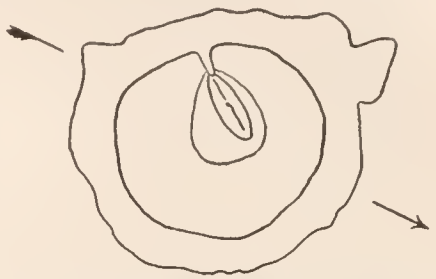


FIG. 1.—Schematic representation of embryo and vesicle. × about 4.5. From photograph, plate I, fig. 1, and reconstructions. The arrow indicates the line of section.

II. THE EMBRYO AND ADNEXA.

The main features of the embryonic anlage are shown in the text-figures 1, 2, and 3 and in the photographs of the model (plate 4, figs. 1 and 2). The general shape of the blastoderm is not unlike that of the Frassi embryo, but narrower and as a whole very much larger. Its dimensions, determined on the reconstructions (× 100), are 2 mm. in extreme length by about 75 mm. in breadth at the widest point. The ventral surface of the embryonic disk presents a very slight ventral concavity in the sagittal plane, while at right angles to this the same surface is for the most part convex, *i. e.*, projecting slightly into the yolk-sac. The dorsal surface is in general more strongly convex, owing to the presence of the prominent ectodermic folds. The amnion above completes roughly the curvature of the yolk-sac below. The anterior extremity of the blastoderm is quite regularly rounded and, especially on the left side, is undermined by shallow extensions of the exocœlom; the posterior half tapers evenly to a point.

As may be seen in the dorsal view of the model, the appearance of the posterior third of the embryonic disk is quite different from that of the middle and anterior thirds. This posterior part contains the cloacal membrane and about the caudal half of the primitive streak and is the most regularly formed part of the entire blastoderm. The dorsal surface presents here, on either side of the median line, two rather steep, even slopes (plate 3, fig. 1), the left slightly more extensive, which extend from the region of the cloacal membrane and primitive streak to the attachments of the amnion laterally. The primitive groove appears in the model simply as the central, deepest portion of this valley-like area. The ventral surface of the region in question is strongly convex from side to side. Distinct primitive folds can not be made out.

The central third of the embryo includes the anterior half of primitive streak and the head-process region in front of it. Most conspicuous here are the two large folds of ectoderm which extend from near the middle line to the attached border of the amnion; the fold on the left side is broader and more regular than that on the

right. Separating these prominent folds (plate 3, figs. 2 and 3) lies, in their caudal halves, the anterior end of what we may call the primitive groove, here very deep and narrow. The groove between these folds in their cephalic portion is much shallower and finally lost. Near the posterior end of this shallower groove, which is continued forward without distinct interruption from the primitive groove, but in a plane slightly to the right, lies the minute dorsal opening of the archenteric canal—so-called chordal canal—slightly to the left in the bottom of the groove.

The anterior third of the blastoderm is in general slightly convex, but its surface is broken up by many small, irregular folds to which one can attach no significance. It is certain that the ectoderm in the anterior half of the embryo has suffered more distortion than any other part. The result has been an obliteration, as far as they may have been indicated, of the early medullary folds anteriorly, coupled with what seems to be their accentuation and prolongation posteriorly. That these last-mentioned folds, occupying the center of the blastoderm, have anything to do with the medullary folds is, considering the stage of development, very doubtful. The posterior ends of these folds, especially on the right, have a remote resemblance to the caudal lobes of a later date.

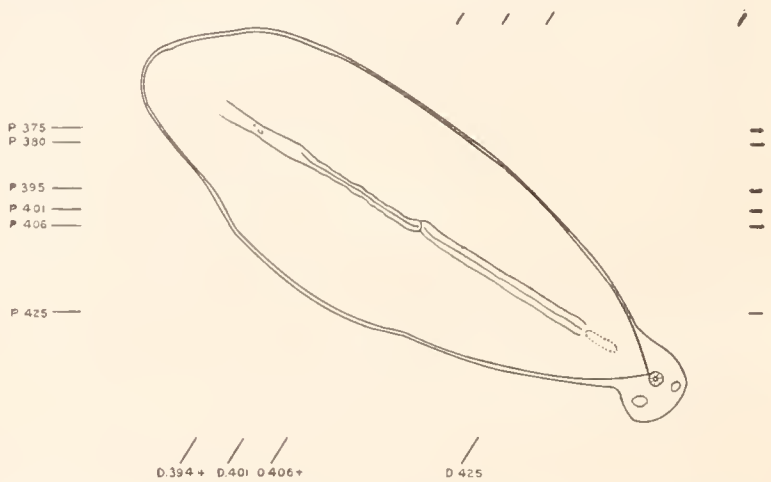


FIG. 2.—Blastoderm and axial structures in dorsal view. × about 40. For more exact details of the head process see text-figures 4 and 5. The location of the cloacal membrane is indicated by dotted outline. Close to the posterior tip of the amnion is the allantois; in section and near it, in the body-stalk, are sections of two vessels. The marginal lines have reference to the location from which were taken the photographs (P.) and drawings (D.) shown on plates 2 and 3.

The amnion lies close to the embryonic ectoderm anteriorly, while farther back it is lifted high above it by being incorporated in the body-stalk. Any indications of the presence or recent disappearance of an amniotic duct as noted by Grosser (1913) and Strahl and Benecke (1910) are wanting. This amniotic duct may very well be one of those instances of embryonic variation referred to above, not only variable but quite possibly very transient, and in this same category may be placed a peculiar feature of our embryo to which we would here draw attention. As can be seen in the accompanying illustrations, the caudal tip of the amnion lies in very close proximity to the allantois, a short distance above the connection of the latter with the yolk-sac. In at least two sections there is a very distinct though tiny, narrow outpocketing of the amniotic cavity toward the allantois (not shown in the figures). Here the epithelium of the amnion is of a low cuboidal type in contrast with its squamous character in the immediate vicinity. There is no connection

between the cavities of the amnion and allantois, but their epithelial cells fuse into a single mass over a small area of contact. The lumen of this amniotic diverticulum, which is also very short, is only a few microns in diameter, tapering slightly toward the allantois. This structure may well be compared with the secondary connection set up between the same cavities in certain reptiles, the canalis amnio-allantoideus of Strahl (Schauinsland, 1902).

Histologically the amnion is composed of two layers of cells which are generally frankly squamous. The transition of the ectodermic cells to the flattened type is as a rule quite abrupt. In certain places, however, the cells, near the attached border of the amnion, are cubical and become squamous only at some distance from the line of reflection. The cells of the mesodermic layer of the amnion have slightly smaller, more densely staining nuclei and seem to present a clean, even surface to the exocœlom. Throughout most of the membrane its two layers are in close contact, often almost indistinguishable, but along the borders there is frequently a considerable space between the two, across which run numerous irregular cell-processes connecting the ectoderm and mesoderm but apparently belonging rather to the latter. Scattered mesodermic cells in the interval between the layers are quite common. Posteriorly, where the membrane is cut tangentially, the ectodermic elements appear polygonal in outline, with large, pale nuclei which almost fill the cell.

In the consideration of the embryo proper we shall begin with the most posterior structures and gradually work forward. In like manner we shall endeavor to separate the following observations from the speculations which they invite.

Mention has already been made of the indications of a canalis amnio-allantoideus in the caudal extremity of the amnion. A short distance in front of this, in the axis of the blastoderm and at the posterior end of the primitive streak, lies the cloacal membrane. As shown in figures 2 and 3, it measures about 0.12 mm. in length. This measurement and likewise the figures are at best approximations, maximum limits in any case, since it is difficult to determine the exact line, if there be one, between the membrane and the primitive streak. There is in the cloacal region a well-defined groove in the ectoderm, less conspicuous, however, than the primitive groove with which it is directly continuous. In certain sections it is quite evident that the ectoderm and entoderm are in immediate contact, the mesoderm being at some little distance. In other sections, largely on account of the irregular lower surface of the ectoderm, the picture is very much like that of the primitive streak. The conditions found here in the cloacal membrane are such as would be expected from the gradual and not entirely regular transformation of the streak into the membrane. All that is required is an arrest of mesoderm formation and the subsequent separation of the upper and middle germ-layers. The entoderm below is a perfectly distinct layer the cells of which have nuclei larger and paler than those of the other layers. The condition of the ectoderm is such that the real character of its cells can not be made out. Its free surface is of course distinct, but the lower surface is often markedly irregular and frayed out. In the region under discussion at present it is undoubtedly of the columnar type, in most places, if not everywhere, pseudostratified with one, two, or occasionally three layers of

nuclei. Farther back and laterally the ectoderm is frankly one-layered, with low columnar or even cuboidal cells. Throughout most of the embryo, however, the arrangement of its nuclei in several layers, the character of its lower surface, and its often irregularly varying thickness make impossible any definite statements as to its real structure. Cell boundaries are not commonly visible.

FIG. 3.—Median sagittal section of embryo, yolk-sac, body-stalk, allantois, and adjacent chorion, slightly schematized, about × 40. The primitive streak and head process are represented as if both lay in the same plane. The chorionic villi are quite diagrammatic, their connections with each other not being indicated. Two "funnels" in the body-stalk, further details in text. Marginal lines as in fig. 2.

The primitive streak (figs. 1, 2, and 3; plate 2, fig. 1; plate 3, fig. 1) is very long in this embryo, making up about one-third of the axis of the blastoderm, the center of the latter being just in front of the anterior end of the streak. Its length, measured from the anterior limit of the cloacal membrane to the dorsal opening of the archenteric canal, is about 0.65 mm. In position it is not exactly central, but is displaced a trifle to the right. The primitive groove is well defined throughout, the continuation, as noted above, of its anterior end passes slightly to the right of the opening of the archenteric canal and the beginning of the head process. This condition, in which the primitive streak and its head process do not lie in the same sagittal plane, is not uncommon in a variety of forms, and apparently the head process is usually on the left, as in this case. The anterior limit of the streak is of course

easily determined, and our reason for assigning the posterior limit is that at this latter point there is not only a conspicuous connection of mesoderm and ectoderm, but also a rather sudden separation of the entoderm from the cell-mass just above. This cleft between the entoderm and the primitive streak is present throughout its caudal half, while anterior to this the entoderm lies very close to the newly formed mesodermic elements.

The primitive groove is best defined at its posterior end, where it appears as a sharply outlined groove between the more gentle slopes of the ectoderm on either side. Farther forward, where the high ectodermic folds are found, this fine median furrow can not be distinguished, being simply the bottom of a deep, narrow trough. In its most caudal part the primitive groove possesses a narrow, flat floor bounded by perpendicular walls of distinctly greater extent. The breadth of the floor may be estimated at about 0.01 to 0.015 mm. This dimension is accentuated in the photographs on account of the obliquity of the sections. Followed forward, the groove varies in shape and width, its floor soon disappears, and it is finally lost in the general slope of the embryonic ectoderm. In the primitive groove the outlines of the ectodermic elements, free surface, and cell boundaries are more distinct and the arrangement of the nuclei is rather more regular than elsewhere.

In those places where there is a well-defined floor one can sometimes see, in one side of the floor, a deep, distinct secondary groove, usually on the right. A few sections show a definite lipping of the primitive groove, *i. e.*, the lateral wall; this being observed only on the left side, bulges into the groove, forming a small recess between the floor and the wall. These peculiar conditions occur only near the posterior end of the primitive groove. The ectoderm of the groove presents 2 to 3 or 4 layers of nuclei and is thickest in front. The floor is often composed of but a single layer of low cells, remarkably thin in places but always intact.

From the walls of the primitive groove, much less conspicuously from its floor, where this is well marked, the ectoderm is continued directly into the mesoderm lying laterally, while the floor of the groove, often much thinner than the side-walls, is widely separated from the entoderm, in which space occasional free cells may be seen. There are in a few places interruptions in the transition from ectoderm to mesoderm, probably artefacts due to the loose character of the latter layer. The width of the primitive streak, *i. e.*, of the zone of proliferation of mesoderm, is about 0.05 mm. Towards its anterior end this zone gradually becomes more massive, the connection between ectoderm and mesoderm more extensive, and the entire median line between ectoderm and entoderm becomes filled with closely packed mesodermic cells. At the very anterior end of the streak, just behind the primitive node, the lower germ-layer is again widely separated from the cells dorsal to it. Everywhere, however, in the primitive streak can the entoderm be seen as a distinct, definite cell-layer.

The mesoderm on either side of the middle line forms a well-defined, loosely cellular stratum of slightly varying thickness. It is continuous laterally and posteriorly with the outer layers of the yolk-sac and amnion, anteriorly with the mesoderm on either side of the head process, and mesially with the ectoderm of the primitive

streak. This layer of cells is thinnest behind and lies uniformly close to the entoderm, to which its cells are attached by numerous fine processes. The constituent cells vary considerably in size and shape; most of them possess a number of larger or smaller, partly anastomosing processes, while some seem to have a smoothly rounded cell-body. One finds quite generally a fine, sharp line on that surface of the mesoderm toward the ectoderm, very much like a basement membrane of connective-tissue origin, the membrana prima of von Spee. There is no indication anywhere of an arrangement of the mesodermic cells in two layers, as has been repeatedly described in the primitive-streak region.

The majority of the mitotic figures observed in this specimen are found near, or at a short distance from, the primitive streak; by far the greater number of these occur in the ectoderm and mesoderm, especially in the former; only rarely are they

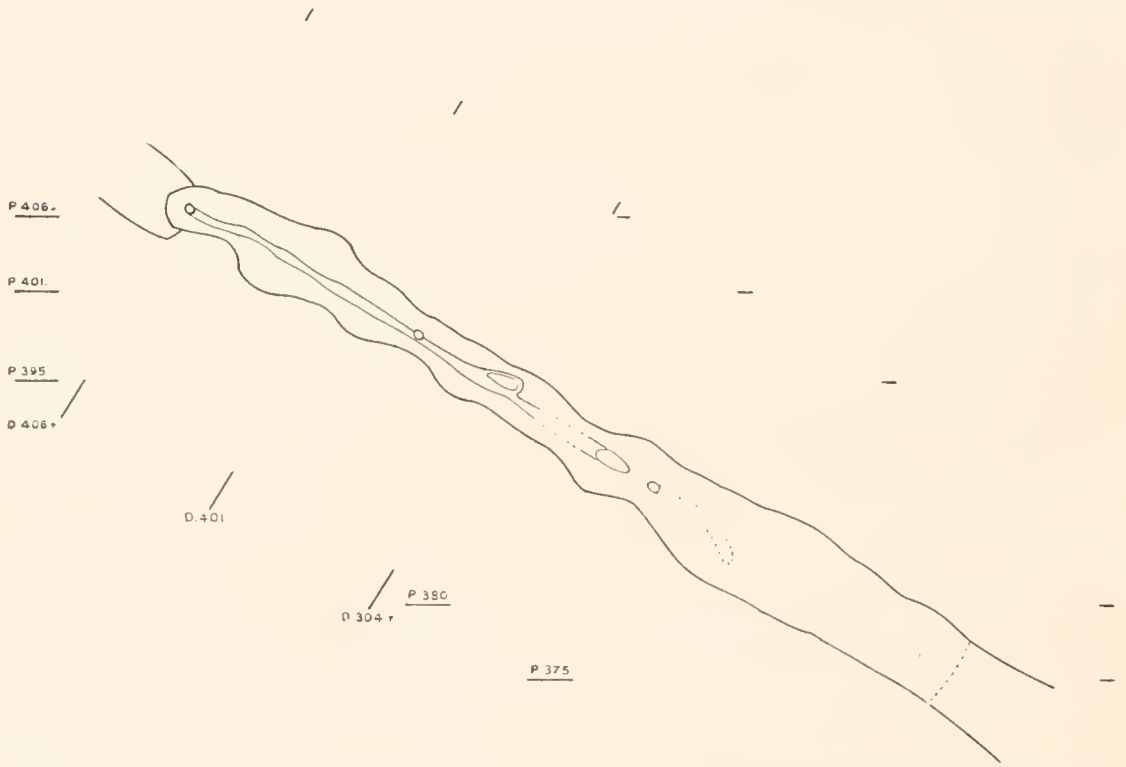


FIG. 4.—Head process and completion plate. Dorsal view, but in the plane of the sections (*i. e.*, some foreshortening. *Cf.* fig. 3). On the left is the anterior end of the primitive streak; immediately anterior to this is the dorsal opening of the archenteric canal. The four ventral openings of the canal are seen in the center of the figure; dotted lines indicate that the lumen is indistinct or doubtful. The small dotted ring near the anterior end represents a very doubtful cavity, and just beyond this is shown the line where the entoderm becomes a distinct layer. Marginal lines as before.

seen in the entoderm. In those cases in which the axis of the spindle can be determined it is found in nearly all instances parallel to the surface of the ectoderm or mesoderm and at right angles to the median line.

As mentioned above, the anterior part of the primitive groove is but the bottom of a deep, median furrow in the blastoderm. This furrow becomes rapidly wider and

is continued some distance farther forward, but distinctly to the right of the head process, where it gradually fades out. Here, at the anterior end of the primitive streak, is the primitive or Hensen's node. There is, strictly speaking, no real node, knot, or distinguishable enlargement at this point, and nothing to indicate any separation between the groove and the archenteric canal. Immediately caudal to the beginning of the canal the ectoderm becomes thinner and there appears a wide interval between the entoderm and the last of the primitive streak dorsal to it.

Lateral to the node, or better in it, *i. e.*, in the walls of the first part of the canal, the ectoderm and mesoderm are in broad connection. Just anterior to the node the head process has freed itself from the overlying ectoderm, is continuous with the mesoderm on either side, and fused with the entoderm below. The posterior ectodermic opening of the archenteric canal is very minute, being only about 0.005 mm. in diameter (plate 2, fig. 2; plate 3, fig. 2). The actual opening on the surface can hardly be made out, since it is bounded only by the slightly staining cytoplasm of the surrounding cells, the nuclei of which, in contrast to other regions, are here at a

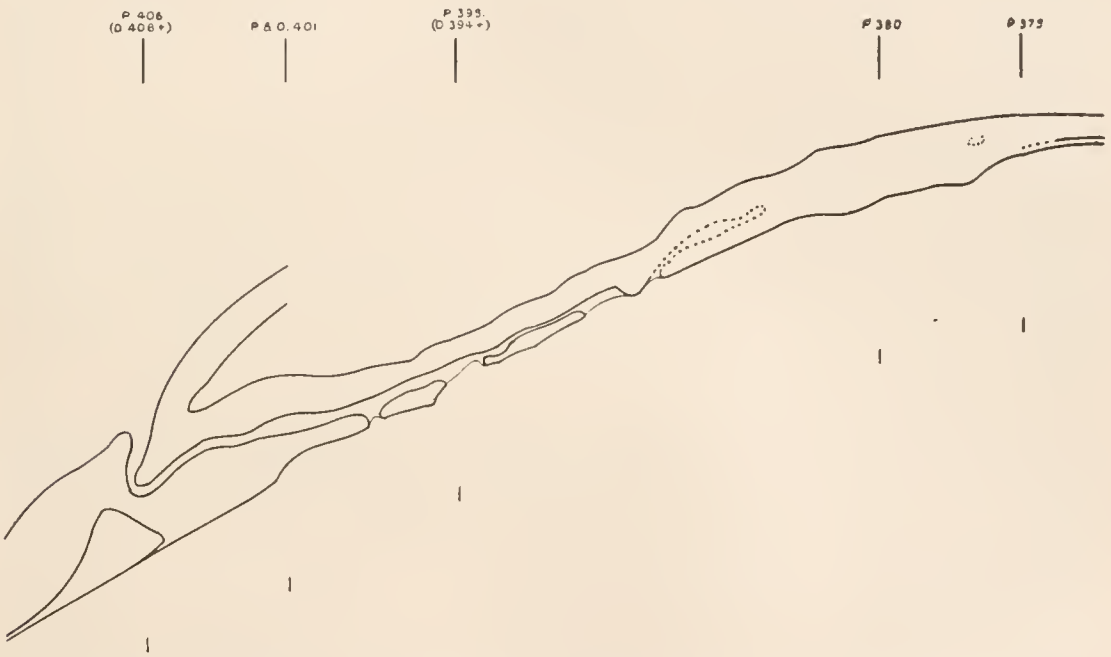


FIG. 5.—Head process and completion plate. Right lateral view. The primitive streak, on the left, and the posterior end of the archenteric canal are represented as being in line with the more anterior structures. Other explanations under fig. 4.

greater distance from the free surface. From this point the canal passes directly ventrad through the substance of the primitive node, turns forward and to the left, and again forward in the line of the head process (figs. 2, 3, and 4). It is at the node, and here only, that the three germ-layers are fused with each other (plate 3, fig. 5).

Extending cephalad from the primitive node in the axis of the blastoderm is the head process of the primitive streak (figs. 1 to 5). This structure, including the completion plate in front, is slightly longer than the primitive streak, measuring about 0.75 mm. in length; its diameter is variable, but in general gradually increases from behind forward. The posterior half, or head process proper, varies in width from 0.03 to 0.05 mm., its lumen from 0.006 to 0.01 mm., while the length of its lumen, the archenteric or canal of Lieberkühn, is 0.34 mm. The average breadth of the completion plate is about 0.06 mm.

The head process is an axially placed, hollow, cylindrical mass which, at its origin in the primitive node, is directly continuous with the superficial ectoderm and the substance of the primitive streak, as well as with the mesoderm on either side. It very soon becomes free from the ectoderm above and fuses with entoderm below; its lumen, which is at first nearer the dorsal surface of the process, takes up a central position, while at the same time the dorso-ventral diameter diminishes somewhat. In considering this structure we shall begin at its posterior end, at the point where it has just disengaged itself from the surface ectoderm. It appears here in section as a roughly pyramidal or wedge-shaped mass projecting well into the space below the ectoderm. Sharply limited above, this mass is fused at its base with the entoderm and mesoderm. The lumen is yet eccentrically placed; the cells dorsal to the lumen are much fewer in number, more deeply staining in their cytoplasm, and have a more epithelial arrangement than those between the lumen and the yolk-sac. These latter cells are much more numerous, more irregularly massed together, and are quite indistinguishable from the neighboring mesodermic and entodermic elements. A few sections in advance (plate 2, fig. 3; plate 3, fig. 3) the head process is rather lower and distinctly broader, its free outlines more curved, while its cavity has increased in size and lies about the center. The cells which bound the archenteric canal dorsally are frankly epithelial; their nuclei are nearer the base of the cells, while the cytoplasm is deeply stained.

The cells ventral to the lumen show no definite arrangement; they stain only faintly and no layer of entoderm can be made out beneath them. The mesoderm is directly continuous with both these groups of cells, but rather more definitely, on account of their staining reactions, with the cell-mass below the canal. The ventral surface of the head process is, near its posterior end, concave from side to side and at about its margins the entoderm can be recognized as a separate cell-layer. The thinning out and eventual loss of the floor of the canal is apparently due to the rearrangement of the cells here (*cf.* fig. 5), there being no evidence of a corresponding loss or destruction of cells. We have in these two distinct cell-groups, dorsal and ventral to the lumen of the head process, the plaque notochordale and plaque lécithoentérique respectively of van Beneden (1899). To these we shall take occasion to recur later. Near its posterior end, where the lumen is more dorsally placed, the cells of the plaque notochordale are only about half as numerous as those of the plaque lécithoentérique; at the point shown in the illustrations they are approximately equal in number.

A little anterior to the sections just mentioned is found the first ventral opening of the archenteric canal. It is very small, located entirely in one section, and appears as a narrow passage connecting the canal with the cavity of the yolk-sac. Just beyond this is the second ventral opening (plate 2, fig. 4; plate 3, fig. 4), very large, mainly on the left side, and limited dorsally by the beautifully epithelial notochordal plate. The epithelial cells of the plate are here columnar, their nuclei are slightly nearer the base than the free surface, and their cytoplasm stains rather intensely. In spite of their character these cells are not to be separated laterally from the adjoining mesoderm and entoderm. In the sections which follow, the notochordal plate varies considerably both in breadth and distinctness. The canal likewise is not always well defined; its floor varies in thickness or may even appear deficient. It can be traced forward, however, to the third and last definite ventral opening, where again the notochordal plate is very conspicuous, while in the floor appear a few cells, some of which may be free. This last portion of the head process hardly projects above the level of the neighboring mesoderm.

Anterior to the head process and continuous with it lies the so-called completion plate, the Ergänzungsplatte (des Urdarmstranges) of Bonnet. Its posterior limit may be placed just in front of the third and most anterior opening of archenteric canal, while at its opposite extremity it is gradually lost as the two lower layers of the blastoderm becomes distinct. Its length, with the limits just noted, may be taken as about 0.4 mm.; its width, averaging about 0.06 mm., is greater than that of the head process proper. In structure it differs very markedly from the typical head process just described. The transition between the two appears to be gradual, at least so far as can be determined on a transverse series. On following the sections forward it is seen that the conspicuous dorsal cells (notochordal plate) rapidly lose their epithelial character, and the lumen (which was such a prominent feature before) becomes very doubtful if not actually wanting (indicated by the dotted outlines in figs. 3, 4, and 5, solid line in fig. 2). At the same time there is a gradual but not uniform increase in the breadth and thickness of the plate until it reaches nearly twice the dimensions of the head process, bulging below into the yolk-sac and above into the space between the ectoderm and mesoderm. Along its lateral borders, which are never sharply marked, it is directly continuous with the mesoderm, as this layer is with the head process farther back. At its anterior end it is gradually lost in an ill-defined, axial condensation of mesoderm, and very soon this also disappears. Structurally the completion plate is made up of a rather closely packed mass of cells in which no details can be made out. The entoderm beneath does not lose its identity to quite the extent which it does in the head-process region, but still can hardly be recognized as a distinct layer. Toward the anterior limits of the plate the entoderm appears as a definite layer of large, thick, almost cuboidal cells. At certain points there are indications of a sort of doubling in the plate, due to the presence of a shallow furrow on its dorsal surface. Here, and also where this feature is not apparent, the faintly defined cavity lies distinctly on the right side. Far forward, near the extremity of the plate, there is again a faint indication of a small cavity. One peculiar feature of the plate is the presence in or between the cells

(one can not in this case say which) of numerous rather large, rounded, intensely staining granules, very similar to those described by Bonnet (1901) in the completion plate of the dog. Although a few of these granules can be seen in other locations, as also figured by Bonnet, they are by far most numerous and conspicuous in the completion plate.

The variations in size of the head process and completion plate, especially as regard their breadth and the roughly corresponding variations in the lumen, are shown in figure 4. Although such variations are recorded (*cf.* Rabl, *l. c.*, Taf. IV), they are unusually distinct and regular in this case. What significance may attach to them we can not say. They seem too small to correspond with the future segmentation of the mesoderm lateral to them, and we have been unable to discover any special features in this mesoderm, such as possibly more active proliferation of cells in relation to the enlargements, either opposite or between them.

The mesoderm in the anterior half of the blastoderm is essentially the same as that which we have already described; far anterior it becomes very thin.

Any indications of a folding off of the embryo, of a proamnion, or buccopharyngeal membrane are wanting.

The structures thus far considered comprise the essential features of the embryonic anlage. Nowhere, as far as we can make out, is there any sign of future segmentation, and nowhere in the embryo are there either blood-vessels or blood-cells; but at the very anterior end of the embryonic disk there occur a number of prolongations of the exocœlom under the embryonic ectoderm. These exocœlomic diverticula have a very small, distinct, but quite irregular lumen lined by cells similar to those on the neighboring yolk-sac and amnion. They appear as rather long, irregular, tubular ingrowths which take their origin from the exocœlom at the point where the mesoderm of the amnion and yolk-sac meets the embryonic mesoderm. The two anterior diverticula arise in the shallow groove under the anterior edge of the blastoderm. Of these ingrowths there can be made out about four, two on either side. The anterior pair, longer and more distinct, reach nearly to the middle line. Of the posterior pair the right is very short, while the left runs parallel to and just within the margin of the blastoderm. Judging from their location, they might stand in some relation to the future pericardial cœlom.

THE YOLK-SAC.

Only approximate dimensions can be given here on account of the folding, partial collapse, and a somewhat extensive tear near the anterior end of the sac. We may estimate its antero-posterior measurement at about 2.5 mm., in a dorso-ventral line at about 2 mm., and a little less than this latter figure from side to side. As seen in the illustrations (fig. 3; plate 1, fig. 3; plate 4, figs. 1 and 2) it is still very large as compared with the embryo projecting well beyond it on all sides, particularly in front and on the right. Originally it was doubtless quite regular in shape. The surface of the sac is for the most part quite smooth and regular, but over a certain area on the fundus anteriorly it has the characteristic uneven, nodular appearance arising from the early blood formation in this region.

The epithelium lining of the umbilical vesicle varies considerably in different parts. In the axis of the embryo the entoderm consists of flattened cells which form a distinct layer except in the region of the primitive node, head process, and completion plate. Elsewhere in the embryo the entoderm is a definite layer of flattened elements whose nuclei stain less deeply and are possibly a trifle larger than those of the mesoderm just above. In the immediate vicinity of the embryo the walls of the yolk-sac consist of two thin layers of cells, usually closely applied to each other, particularly toward the anterior end, but between the layers occur scattered mesoderm cells which are much more numerous in the posterior part of the sac. Farther from the embryo the entoderm cells gradually become thicker, their cell-bodies become more definite, and they take the stain more readily. Over the fundus the lining cells are in general cubical, with well-marked boundaries. Here there is extensive formation of blood-cells and blood-vessels which we shall not discuss at present, except to say that there is no connection between these vessels and those of the body-stalk. In the fundus of the sac there occur two small outpocketings of the entoderm into the covering mesoderm (fig. 3). In these diverticula the epithelium is higher and its cells larger than elsewhere.

THE ALLANTOIS.

The allantois is given off from the yolk-sac a short distance behind the cloacal membrane. It immediately enters the body-stalk, running at about right angles to the plane of the embryo and, as noted on page 118, is at one point in intimate contact with the amnion. Its length, without reference to its slightly curved course, is about 0.65 mm. The lumen is largest just above its origin in a small funnel-shaped depression in the yolk-sac. Its free, slightly coiled extremity has a cavity almost as large as at its origin, while between these the duct and its lumen are narrowest. The average outside diameter is about 0.04 mm. Its walls are composed of low columnar cells containing large, densely-staining nuclei. As it appears in the sections the duct lies in a large space due to the shrinking away of the surrounding tissue.

THE BODY-STALK.

The body-stalk is short and distinctly flattened from side to side. Embedded in the loose mesenchyme of which it is composed are the allantois, the posterior part of the amnion, and numerous vessels filled with nucleated blood-cells. No attempt has been made to reconstruct or learn the exact disposition of these channels. In places their walls seem to be deficient and they take on the character of the unlined spaces. The outer covering of the body-stalk is a rather prominent mesothelium, which is best marked near the embryo and also on what we may call the posterior aspect of the stalk. Toward the chorion this covering stops abruptly, at a varying but short distance from the attachment of the stalk, and there also appear to be deficiencies in this covering, especially on the anterior surface of the stalk. From this mesothelial layer there are a number of more or less definite ingrowths, a few of them forming quite distinct "funnels." Other findings in the body-stalk are the unlined spaces, angiocysts, and angioblast cords described by Bremer (1914).

Connections between these last-named structures and the mesothelial ingrowths are not especially in evidence, but we have not gone into a detailed study of them in this respect. The occurrence of similar conditions in the wall of the yolk-sac we would not like to exclude, because the histological pictures are here less satisfactory than anywhere else in the whole specimen.

III. THE CHORION (MICROSCOPIC) AND EXOCŒLOM.

Examination of the sections (plate 1, figs. 3, 4, and 5) shows that the villi in the equatorial zone are much more developed, longer, larger, more numerous, and more richly branched than on the two flattened poles of the vesicle. The mesodermic portion of the chorionic wall is a thin, fairly uniform stratum, the ragged exocœlomic surface of which is in marked contrast with the same surfaces of the embryonic structures. Within the larger villi, even far from the embryo, are found numbers of open spaces, some of the smaller having a fairly distinct endothelial lining. Some of the unlined spaces in the wall of the sac are very large, and they may be brought about in part or at least accentuated by the pulling away of the mesodermic from epithelial constituents of the chorion which is in evidence almost everywhere. Occasionally short strands resembling the angioblast cords are seen, even in the bases of the villi, but these and undoubted vessels in the villi are by far most frequent near the attachment of the body-stalk.

The villi possess a loose mesenchymal core which in the shorter ones extends quite to their free ends, while in the longer and larger equatorial villi this core is not so extensive. The inner layer of the epithelial covering of the villi and also of the chorionic wall is made up of distinct cellular elements, polygonal in outline and varying from thick squamous to low cuboidal, constituting the cytotrophoblast on the layer of Langhans. Cell-boundaries are here uniformly distinct, and both the cytoplasm and the nuclei stain more lightly than the same parts of the overlying syncytium. Often the line between the Langhans layer and syncytium is very sharp, again decidedly vague, while in numerous places either layer may be so reduced as to seem the only covering of the mesodermic core. Most frequently it is the syncytial layer which is so markedly thinned or apparently absent. The fact that the line between these two layers can not always be seen, and the occurrence in the deeper portions of the syncytium of what seem to be indistinct cell-boundaries, would point to the close genetic relationship of the two layers. Distally the cellular layer of the villi passes over into the cell-columns by means of which the villi are extensively united. This is especially conspicuous in the case of the equatorial villi, among which are also found extensive irregular masses, the trophoblastic cell-islands, which on the surface toward the ovum gradually merge into the cell-columns of the villi. These cell-islands are composed of large, very pale cells with distinct boundaries and large, pale nuclei. The constituent elements are for the most part irregularly polygonal, but they may take on an elongated, spindle-like form, as if actively drawn out. A faint vacuolization is not infrequent. In many places, but most marked in the neighborhood of the embryonic attachment, these cellular masses form practically an inclosing shell over the intervillous spaces beneath.

The syncytium, or plasmoditrophoblast, over the vesicle wall and the bases of the villi consists of a thin layer of slightly varying thickness, but as a rule thinner than the cellular layer beneath it. Both its cytoplasm and nuclei stain very densely. Traced outward upon the villi, the syncytium rapidly thins out on the cell-columns and soon disappears. The largest syncytial masses are found in the equatorial zone just outside the cell-islands. Here it forms large, often extensively vacuolated or spongy masses which can not always be definitely separated from the cell-islands. Scattered through the intervillous spaces, some of them close to the wall of the vesicle, are free syncytial masses of every possible size and shape. The nuclei vary widely in number; they may be small and stain quite densely, or large and pale, and this in the same bit of syncytium. "Prickle processes" are seen quite distinctly on some of these masses and their protoplasm is often very finely vacuolated. Smaller fragments of syncytium often lie in shallow pits or excavations in the cell-islands or trophoblastic columns. These masses are often very small, with one or more nuclei, and are only very lightly stained. Here again there seems to be a direct transformation of cytotrophoblast into plasmoditrophoblast. If there are evidences of cell-division in the chorion they have so far eluded us.

The amount of maternal blood in the intervillous spaces varies considerably in different localities. In a few places it is very abundant, in others almost wanting. It is most plentiful on the flattened poles of the ovum, where the villi are fewer and shorter and where the cell columns and islands and syncytial masses are least in evidence. It would appear as if the anastomosing cell-columns around the equator of the ovum had prevented the entrance of maternal blood, except very indirectly through the more distant intervillous spaces. That the blood should have drained out more readily from these deeper spaces, many of which are closed externally by the remains of the trophoblastic shell, seems quite improbable. Over much of the ovum externally is a layer of clotted blood in which leucocytes are more numerous than in the blood in the intervillous spaces.

In concluding this account of the chorion mention may be made of a small cyst-like structure faintly seen on plate 1, figure 3. It is composed of tissue to all appearances like the mesoderm of the chorion and lies close to, but seemingly not in connection with, the vesicle wall. No indications of a chorionic duct have been encountered.

Concerning the magma in the exocœlom, it will be recalled that upon gross examination of the ovum a few fine strands were observed connecting the yolk-sac and chorion. At that time it could be seen that traction upon these strands was not without effect upon yolk-sac. In the sections there can be found only some ragged wisps of a finely fibrillar nature, which at various points grade insensibly into irregular clumps of a finely granular or fibrous character extensively present in the cavity of the vesicle. In a few places where the larger strands have an attachment to the chorion there occur very intensely staining nuclei. Where best developed the fibrils are very conspicuous; they form loose bundles and stain very dark with hematoxylin. Over the amnion and the yolk-sac near it is a very thick, condensed layer of a finely granular texture (plate 2, figs. 5 and 6).

IV. GENERAL DISCUSSION.

The embryo which we have just described represents an extremely interesting and instructive stage in the ontogenesis of man. In it are found as many important features of early development as could well be expected in one and the same specimen. Besides presenting so may typical and classical features, it has the added advantage of showing them on an unusually large scale. This size, as already mentioned, may be considered simply as a variation; accentuated it may be by unknown influences. It is well known that certain developmental stages are quite ephemeral; that there is further a greatly varying susceptibility in different tissues and in these at different times, and herein may lie some explanation of the conditions described above, perhaps an unusual development or late persistence from unknown causes. We may recall here that Rabl makes repeated mention of considerable variations in size, age, and development in the areæ embryonales of rabbits, often insisting that they can not be looked upon as either abnormal or distorted, although offering no explanations. We may quote in this connection his own words (*l. c.*, p. 378; *cf.* also Taf. IV) regarding embryos with one somite: "Da habe ich denn von einer sehr merkwürdigen Erscheinung zu berichten. Ich habe nämlich zwei Arten von Embryonen dieser Entwicklunsstufe beobachtet: die eine war kurz, breit und gedrungen, die andere lang, schmal und schlank." The gist of the above is that we consider our embryo normal, though not typical.

Any discussion of the findings in this embryo naturally revolves around the question of gastrulation and the formation of the germ-layers. We shall not at this time attempt an extended treatment of the subject, but give simply our own interpretation of what we have observed in this particular case. Naturally one should not conclude too much from a single stage, either as to antecedent or later conditions; but every stage must be in harmony with those which precede or follow, and the truth is not always commensurate with the extensivenss of the evidence. On many problems of development this embryo of course throws no light whatsoever, being far too advanced.

As regards the formation of the amniotic cavity and the yolk-sac, we may accept them as currently given. The question of the mesoderm is not so easily disposed of. In spite of its precocious development, we can not yet see the necessity of denying that it may still be, in principle, peristomal mesoderm. Considering the recent attempts of Rabl in this respect and the similar difficulty regarding the entoderm, it would seem to us that the inherent questions of gastrulation and homology should be more definitely disproven before an entirely new and foreign mode of development is invoked.

In the primitive streak we have a closed blastopore, howbeit radically altered. At its anterior end is an opening and what is theoretically at least an invagination, the head process with its archenteric canal. The posterior end of the streak is, in this stage, marked by the cloacal membrane which is later also open, at present in process of formation. Between the two points there is extensive mesoderm formation, as witnessed by the mitoses in this region, peristomal mesoderm. If one were

inclined to carry the comparison still farther, the peculiar features of the primitive groove mentioned on page 121 might be interpreted as an attempt at the formation of lateral blastoporic lips. How much of the mesoderm of the embryo appears first as strictly peristomal we of course can not say.

The consideration of the head process involves also the tangled question of the entoderm. The head process of the primitive streak (Kölliker), l'ebauche de l'archentéron of Van Beneden, Bonnet's Urdarmstrang, or the Mesodermsäckchen of O. Hertwig, is one of the most important features of the area embryonalis. In its formation, and that of the primitive streak, we have the essentials of gastrulation in man; in the cavity of the head process, the archenteric canal (Urdarmkanal), is retained all that is left of the cavity of the primitive gastrula, the archenteron. From this head process are derived, to what extent it is impossible to say, gastral mesoderm, further chorda, for the most part, and (for aught we know) more or less of the entoderm of the gut-tract. From the foregoing it will be clear that we do not agree with Keibel (1910, 1913) and Hubrecht (1905, 1909) in considering the formation of the two-layered stage, ectoderm and entoderm, as constituting the process of gastrulation. That entoderm formed by delamination is essentially secondary or yolk entoderm, the paraderm of von Kupffer, Wenckebach's cænogenetic entoderm, the lécithophor of van Beneden.

To what extent this first-formed layer is concerned in the formation of the digestive tract we do not know; certainly in some forms its rôle is by no means an exclusive one. The fact that this yolk entoderm fuses with the head process but not with the primitive streak is but evidence as to its cænogenetic character. The only support of the views of Keibel and Hubrecht is the supposition that this secondary entoderm is the sole and only source of the gut entoderm. The theory and the entoderm stand or fall together. In the walls of the head process, *i. e.*, bounding the archenteric canal, we would expect to find primary or protentoderm, Bonnet's Urentoderm, the palingenetic entoderm of Wenckebach. If the lumen of the head process is in reality an archenteric canal, then we would expect it to give rise to mesoderm (segmented), chorda, and gut entoderm—and such, with the reservations given above, seems to be the case. If the head process is simply the anlage of the chorda plus some mesoderm (whence the misnomers chordal or notochordal canal, chordulation, etc.), why should it contain a definite although inconstant canal communicating with the exterior; why so much more material than is required for the chorda, and why its fusion and communication with the yolk-sac? The answer is that in the formation of the head process and not in the delamination of the secondary entoderm we have a process which can be designated as gastrulation.

As concerns the derivatives of the head process, the case of the chorda is perfectly clear. At this stage its anlage is contained in the dorsal, epithelial wall of the canal, the notochordal plate. The fate of the ventral wall or floor of the canal, the plaque entérique of van Beneden, is uncertain. It fuses early with the yolk entoderm or lécithophor immediately beneath to form the plaque lécithoentérique. The loss of the floor, from the rearrangement of its cells, results in the confluence of the archenteric canal and the cavity of the yolk-sac. This process is naturally cænogenetic,

since the yolk entoderm and its inclosed cavity are cænogenetic features. There are thus restored the original conditions in which the anlage of the chorda and mesoderm (enterocœle) are situated in the dorsal wall of the gut. To what extent there is any formation of gastral mesoderm from the head process is a question. In any case, even if the mesoderm had a peristomal origin, its continuity with the walls of the canal is sufficient to indicate the interpretation of the latter as potential sources of gastral mesoderm. With the disappearance of the floor of the canal there is ushered in the stage of the so-called intercalation of the chorda in the entoderm. This obviously takes place quite irregularly and the picture is exactly that seen on such a large scale in Reptilia, but clearly marked in many other forms. This stage is shown on plate 2, figure 4, and plate 3, figure 4. To be exact, this is not an intercalation of the chorda in the entoderm. The notochordal plate is in connection laterally not only with the entoderm, but much more extensively with the mesoderm. If one suppose that there may still be mesoderm formed from the borders of the plate (and there is here no evidence to the contrary) it would be possible to raise objection to the use of the term "notochordal plate," since it would contain chorda and gastral mesoderm. Not, however, until there is a definite separation of the plate from the mesoderm and its continuity with the entoderm alone can one speak of an intercalation of the chorda.

The extent, if any, to which the plaque lécithoentérique (Dotterdarmplatte) contributes to the formation of the wall of the future digestive tract is difficult to determine and certainly not to be decided by any one stage. There are a number of facts, however, which seem to point to such a participation. The marked disproportion between the notochordal and enteric plates in the posterior, least differentiated part of the head process and the retention of the former, practically intact throughout its whole extent, indicate unmistakably that there is formed from the primitive node and head process a considerable mass of material which is not expended in the formation of the chorda. If this material be not actually lost, then it must find its way into the mesoderm or entoderm or into both. In view of the large mass of material produced, much greater than that destined for the chorda, and considering also its peculiar mode of development, virtually an invagination, the simplest solution is to suppose that both mesoderm and entoderm are formed from the side-wall and floor of the head process. If the development of gastral mesoderm is small or wanting, so much more material for the entoderm. It may be recalled here that the digestive tract in the embryo is very small below the pharynx and no very great amount of material would be required to form its walls. The fact that in certain animals the primary entoderm is concerned in the formation of the epithelial wall of the gut seems to us very significant. It would seem that the absence of definite evidence that the entoderm of the future embryo is not, in part at least, primary entoderm, is outweighed by the above considerations and by the fundamental homologies which they tend to preserve.

With the head process and primitive streak we have not yet exhausted the possibilities for the discussion of fundamental problems; there remains the question of the completion plate, Bonnet's Ergänzungsplatte, the protochordal plate of

Hubrecht. Our knowledge of this structure is less extensive than that of the region we have just been considering, and our remarks will be correspondingly brief. Bonnet's term is a very fitting one, since the derivatives of the plate are the same as those of the head process and directly continuous with them. For Rabl (*l. c.*, p. 239) the completion plate is simply "das vorderste Ende des in Lecithophor vorgeschobenen Kopffortsatzes oder Urdarmsäckchens." For Bonnet and Hubrecht it is developed from the yolk entoderm independently of the head process. The evidence in this particular case would seem rather to support this latter view. The second view, however, is not so easily reconciled with our ideas of gastrulation as the first, and we shall not carry the discussion farther at this time. Concerning the future of this plate, which has been recognized in a variety of forms and given a variety of names, there is much more unanimity of opinion. There arise from the completion plate in the dog, according to Bonnet (*l. c.*, p. 286): "1. Mesoderm des Vorderkopfes, 2. die Chorda des Vorderkopfes, und 3. ein prämandibulares Darmrudiment. Es bildet dieses Gebiet also thatsächlich ein Ergänzungsstück des Urdarmes, indem es dieselben Derivate wie dieser aus sich hervorgehen lässt." The anterior part of the chorda, which, as compared with that derived from the head process is very short, remains long in connection with the entoderm. The formation of mesoderm is also continued here for some time.

The significance of the apparent cavity formation in the completion plate is a matter of uncertainty. It might be compared with the secondary canals which sometimes appear in the chorda as it separates from the entoderm. One could perhaps look upon them as attempts at the development of an archenteric cavity, or they might conceivably stand in some relation to the rarely appearing head cavities. As far as we can make out, the buccopharyngeal membrane would have appeared close to the anterior limit of the completion plate, with the possibility of the plate contributing in its formation.

From the observations here presented and from the consideration of other human embryos, one may conclude that the essential features of gastrulation in man are directly comparable with the classical features of that ancient and important process. Significant parallels may be drawn between early human ontogenesis and that of many other representative vertebrates. The conditions in man are manifestly simpler and more primitive than in many cases which have been extensively studied, these being often very specialized or aberrant forms. Hand in hand with specialization and advancement there is the appearance in ontogeny of cænogenetic features which always tend to obscure the original picture. If man has retained much that is primitive and generalized, then we should expect to find some expression of this in his earliest development.

Cleveland, Ohio, *September 27, 1917.*

LITERATURE CITED.

VAN BENEDEN, ÉD. Sur la présence chez l'homme d'un canal archentérique. Anat. Anz., 1899, XV, 349–356.

BONNET, R. Beiträge zur Embryologie des Hundes. Erste Fortsetzung. Anat. Hefte, 1901, XVI, 231–332.

BREMER, J. L. The earliest blood vessels in man. Amer. Jour. Anat., 1914, XVI, 447–465.

BRYCE, T. H., and J. H. TEACHER. Contributions to the study of the early development and imbedding of the human ovum. Glasgow, 1908.

ETERNOD, A. C. F. Premiers stades de la circulation sanguine dans l'œuf et l'embryon humains. Anat. Anz., 1898, XV, 181–189.

FETZER, —. Ueber ein durch Operation gewonnenes menschliches Ei, das in seiner Entwickelung etwa dem Peters'schen Ei entspricht. Verhandl. d. anat. Gesellsch., 1910, XXIV, 116–126.

FRASSI, L. Ueber ein junges menschliches Ei in situ. Archiv f. mikr. Anat., 1907, LXX, 492–505.

GROSSER, O. Ein menschlicher Embryo mit Chordakanal. Anat. Hefte, 1913, XLVII, 649–686.

———, Altersbestimmung junger menschlicher Embryonen; Ovulations- und Menstruationstermin. Anat. Anz., 1914, XLVII, 264–283.

HUBRECHT, A. A. W. Die Gastrulation der Wirbeltiere. Anat. Anz., 1905, XXVI, 353–366.

HUBRECHT, A. A. W. Die Säugetierontogenese in ihrer Bedeutung für die Phylogenie der Wirbeltiere. Jena, 1909.

KEIBEL, F. Manual of human embryology. Keibel and Mall, vol. I, 1910.

———. Die Entwicklungsgeschichte der Wirbeltiere. Die Kultur der Gegenwart, III, IV, 2, 1913.

MALL, F. P. On stages in the development of human embryos from 2 to 25 mm. long. Anat. Anz., 1914, XLVI, 78–84.

RABL, C. Édouard van Beneden und der gegenwärtige Stand der wichtigsten von ihm behandelten Probleme. Archiv f. mikr. Anat., 1915, LXXXVIII, 1–470.

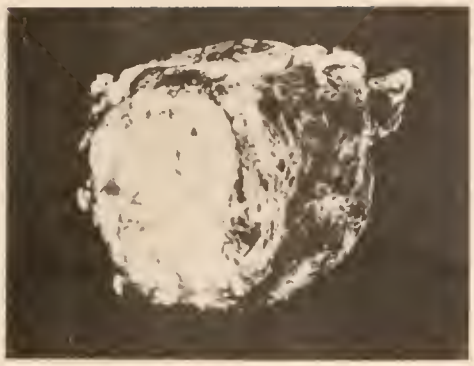
SCHAUINSLAND, —. Article in Hertwig's Handbuch, Bd. I, 1, 1902.

SPEE, F. GRAF. Neue Beobachtungen über sehr frühe Entwickelungsstufen des menschlichen Eies. Archiv f. Anat. u. Entwickelungsgesch., 1896, 1–30.

STRAHL, H. Ueber einen jungen menschlichen Embryo, nebst Bemerkungen zu C. Rabl's Gastrulationstheorie. Anat. Hefte, 1916, LIV.

———, and R. BENEKE. Ein junger menschlicher Embryo. Wiesbaden, 1910.

TRIEPEL, H. Altersbestimmung bei menschlichen Embryonen. Anat. Anz., 1914, XLVI, 385–398.



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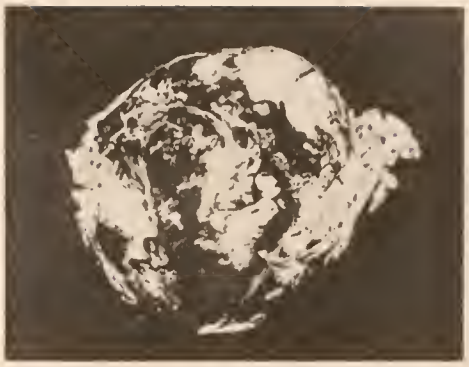

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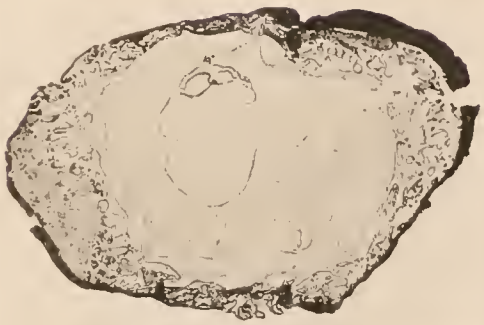

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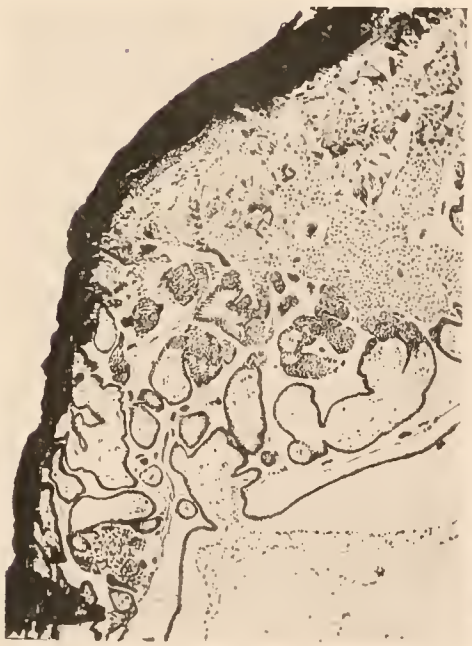

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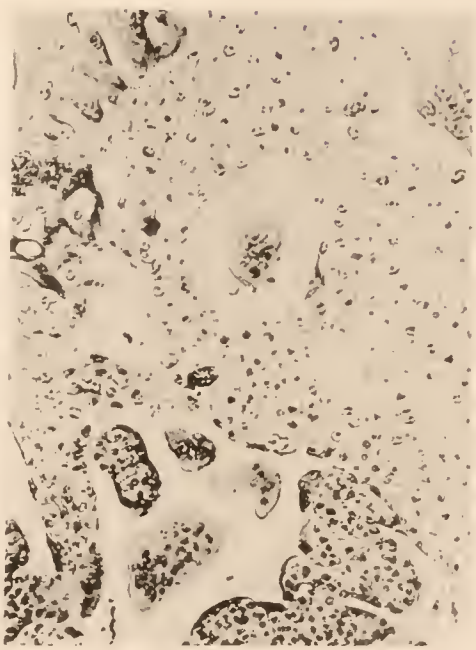
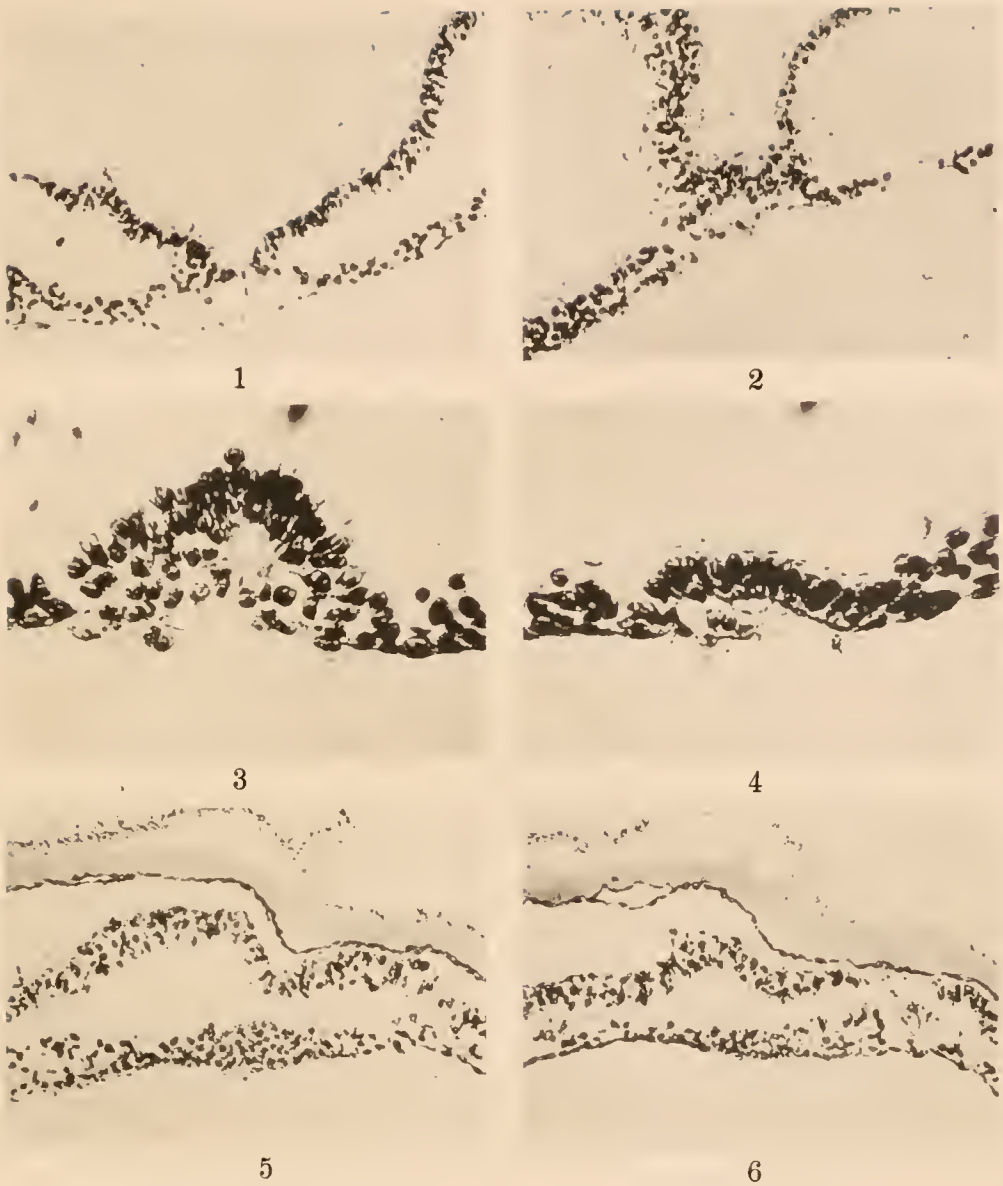


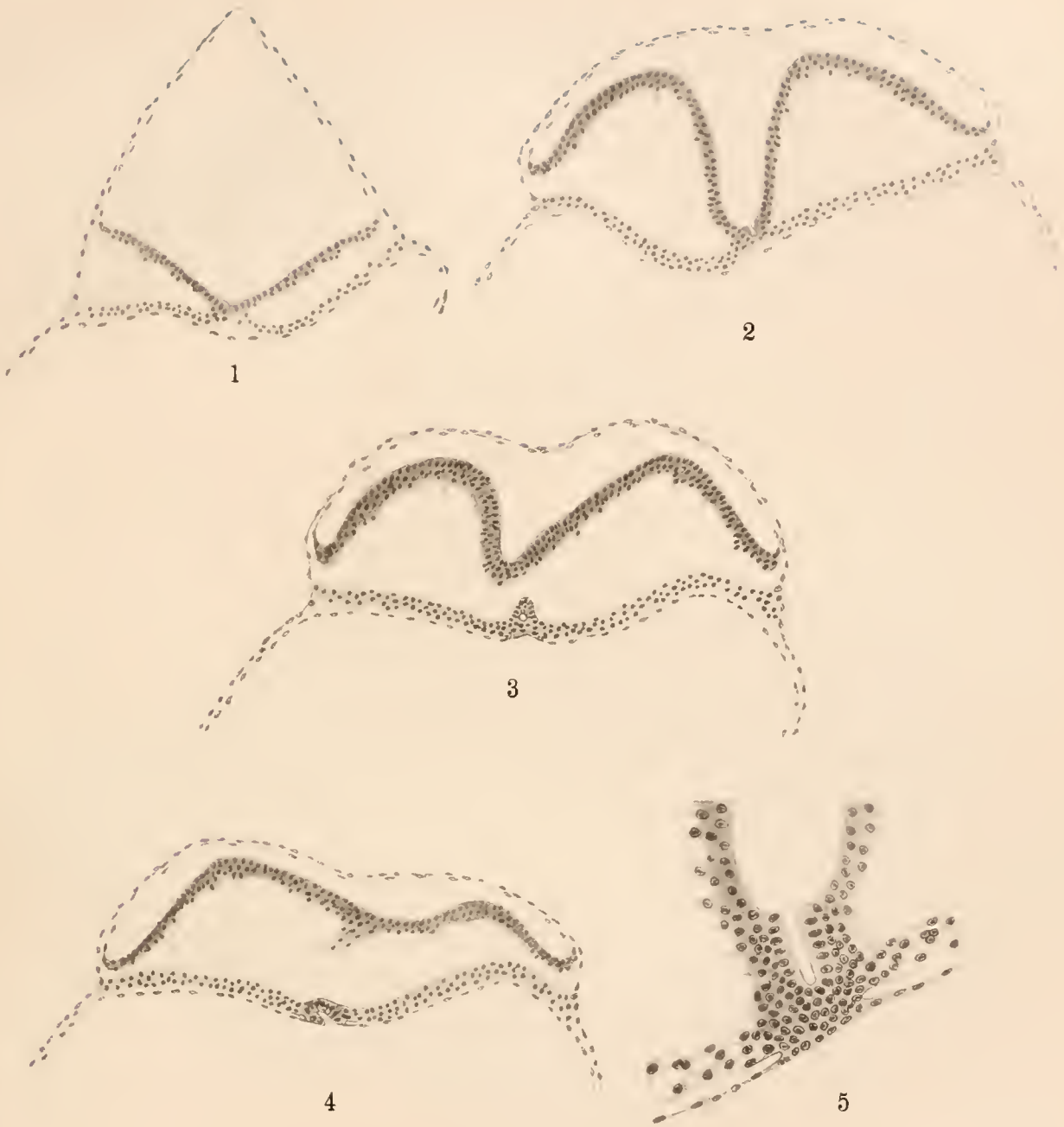
5

1 and 2. Intact vesicle, opposite views. × 4½.
3. Photograph of Section 404. × 9.
4. Detail of Fig. 3 (lower left corner). × 45.
5. Detail of Fig. 4. × 180.



Photographs of sections of axial structures, the location and direction of which are indicated in the text-figures. In all the photographs and drawings the right side of the embryo is on the left in the plates, i. e. all views looking caudad. Dorsal structures are cut slightly more anterior than ventral ones. (Cf. text-figure 3.)

1. Section 425. Posterior part of primitive streak. × 160.
2. Section 406. Most caudal section in which archenteric canal appears. Its location may be recognized by the absence of nuclei. × 160.
3. Section 401. Archenteric canal where largest and best defined. × 400.
4. Section 395. Large (second) ventral opening, "plaque notochordale" very distinct. × 400.
5. Section 380. Completion plate where best developed. × 160.
6. Section 375. Completion plate near anterior limit. × 160.



1 to 5. The following drawings (indicated by D in the text-figures) are obviously reconstructions and therefore somewhat schematic. They would cut the median line at or near the points traversed by the sections shown in the photographs (Plate 2, figs. 1 to 4), and have therefore been designated by the same section number. In other words section (photograph) 425 and drawing (section) 425 would intersect in the median line. Like the photographs they are slightly oblique dorsoventrally.

1. (Section) 425. Posterior part of primitive streak. × 100.
2. (Section) 406 +. Dorsal opening of archenteric canal. × 100.
3. (Section) 401. Typical head process, archenteric canal, "plaque notochordale" and "plaque lécithoentérique." × 100.
4. (Section) 394 +. Large ventral opening, "plaque notochordale." × 100.
5. Idealized cross-section (reconstruction) at the point where all three germ-layers are continuous. Its location, while not indicated, can be made out from text-figure 5.

1

2

1. Dorsal and slightly lateral view of model. × 100. Reduced. On the left the body-stalk is cut across.
2. Left lateral view of model. × 100. Reduced. On the right the body-stalk, cut in the plane of the sections, shows a large vessel; running downward and forward, also in the plane of the sections, a small portion of the amnion has been left. In the embryonic disc are seen the irregular ectodermic folds, its anterior extremity is undermined. The irregularity in the anterior part of the yolk-sac is due to a tear. The upper part of the model has been separated from the lower by sawing through it parallel with the blastoderm; it is this upper piece which is represented in Fig. 1.